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AMMONIUM PERCHLORATE: Effect on Immune Function

Study Report

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AMMONIUM PERCHLORATE: EFFECT ON IMMUNE FUNCTION

BRT 19990524 Study Protocol
Plaque-Forming Cell (PFC) Assay

BRT19990525 Study Protocol
Local Lymph Node Assay (LLNA) in Mice

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INTRODUCTION

Perchlorate may exist in the environment as a part of other compounds such as ammonium, potassium, or sodium perchlorate. Ammonium perchlorate is manufactured as an oxygen-adding component in solid fuel propellant for rockets, missiles, fireworks, and matches as well as for use in analytical chemistry. The perchlorate salts are very soluble in water and can persist for many decades under typical groundwater and surface water conditions.

The present study is part of an initiative to evaluate possible health effects of ammonium perchlorate. There are three (3) major study objectives: (1) to assess immunotoxicity by measuring the effect of ammonium perchlorate on (a) plaque-forming cell (PFC) formation and (b) contact sensitization induced by DNCB, (2) measurement of thyroid hormone (T_4) and TSH levels, and (3) assessment of thyroid histopathology and S-phase labeling.

The effect of ammonium perchlorate in drinking water was evaluated in 14- 90-day studies to assess immunotoxicity. The PFC response is the most commonly affected functional parameter in animals exposed to chemical immunosuppressants (Luster , M.I. et al. 1988. *Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice.* Fundam Appl Toxicol 10:2-19). Luster et al (1992: Fundam Appl Toxicol 18:200-210) assessed 51 different chemicals using the NTP panel and found that with the spectrum of assays utilized, the highest associations with immunotoxic potential were observed for the splenic IgM PFC response and cell surface marker analysis. The PFC response requires functional activity from B lymphocytes, T lymphocytes, and macrophages and as such assesses the functionality of three major components of the immune system (Holsapple, M. In: Methods in Immunotoxicology, Volume 1. Eds. G.R. Burleson, J.H. Dean and A.E. Munson. Wiley-Liss, New York, NY).

Immunotoxicity was further tested to determine the effect of ammonium perchlorate in the drinking water on the ability of mice to generate a hypersensitivity response to 2,4-dinitrochlorobenzene (DNCB), a known sensitizing chemical. The LLNA evaluates the allergic potential of a test substance following topical exposure to both ears by assessing the differential induction of lymphocyte proliferation in the draining auricular lymph nodes of the ears compared to appropriate controls. The proliferative response is measured by quantifying the incorporation of ^{125}IUDR into DNA by the lymphocytes.

Perchlorate is known to disrupt thyroid hormone homeostasis in a number of species via an inhibition of iodine uptake into the thyroid gland. Assessment of thyroid hormone (T_3 and T_4) and thyroid stimulating hormone (TSH) levels in circulating blood is the most sensitive endpoint for the effect of perchlorate on an organism. Caldwell *et al.* (1996) measured T_3,T_4 and TSH hormone levels in male and female rats after 14 days

of exposure to ammonium perchlorate in drinking water. The values for T_3 and T_4 in both male and female Sprague-Dawley rats decreased while the concentration of TSH increased after 14 days. Thyroid organ weights were not measured but histopathology of the thyroid revealed hypertrophy of follicular cells at the highest concentration levels. In the present study, serum samples from control and perchlorate-treated mice were collected after 14 or 90-days, and thyroxine (T_4) and TSH were determined using radioimmunoassay (RIA). All thyroids were processed by routine methods to paraffin as standard cross sections through both thyroids, trachea and esophagus. Paraffin blocks were sectioned at 5 microns and stained with either hematoxylin and eosin or anti-BrdU immunohistochemistry by routine methods. The thyroids were examined microscopically for colloid depletion and follicular cell hypertrophy using the criteria previously developed while reviewing the series of perchlorate studies performed under Department of Defense contracts.

METHODS

Plaque-Forming Cell (PFC) Assay

Test System: The PFC response to SRBC is a sensitive measure of immunotoxicity, detecting alterations on T cells, B cells, and macrophages. It is an accepted method for first tier immunotoxicology testing. BrdU will be added to the water of 5 mice per treatment group during the last 3 days of exposure, as requested by Sponsor, for thyroid cell proliferation assay. Dr. Doug Wolf, USEPA, performed necropsies to obtain organs for histopathology. EPA also collected serum at necropsy for thyroid hormone analysis performed by Dr. David R. Mattie, AFRL/HEST, Wright-Patterson Air Force Base.

Animals: B6C3F1 female mice, 7 weeks of age, were obtained from Charles River and acclimated until the following week prior to use. Mice were housed 5 per cage. The PFC Assay used 10 mice for each treatment group and for the positive control.

Dosing: Mice were dosed daily with ammonium perchlorate in the drinking water for either 14 or 90 days.

Immunization of Animals: Mice were immunized i.v. with 0.2 ml of 2.0×10^8 SRBC in RPMI, 4 days prior to AFC assay. SRBC was titrated for optimum concentration. Positive controls received cyclophosphamide (CP, 15 mg/kg i.p.) for 4 consecutive days starting on day 10 of the 14 day exposure group and day 86 of the 90 day exposure group.

Food and Water: Mice were provided with Purina 5001 Rodent Chow with access to tap water *ad libitum*. Water bottles were weighed daily to determine intake.

Environmental Control: Animals were maintained on 12 hour day and 12 hour night cycles.

Contact Sensitization Induced by DNCB- Local Lymph Node Assay (LLNA)

Test System: The local lymph node assay (LLNA) in the mouse was used as the animal model for determining local lymph node proliferative activity following application of sensitizing agents. Incorporation of ^{125}Iododeoxyuridine (^{125}IUDR) into DNA of lymphocytes isolated from the auricular lymph nodes results from proliferation of the cells after application of a sensitizer to the dorsal side of the ears. Measurement of the ^{125}IUDR uptake by the cells is an objective and quantifiable response.

Dosing: Mice were dosed daily with ammonium perchlorate in the drinking water for either 14 or 90 days. In the 14 day study, mice were placed in individual cages on day 8, appropriate groups dosed with cyclophosphamide, vehicle, or DNCB on days 9, 10, and 11, cyclophosphamide on days 12 and 13, and assayed on day 14. In the 90 day study, mice were placed in individual cages on day 91 and maintained on the appropriate dose level of ammonium perchlorate until day 97, appropriate groups dosed with cyclophosphamide, vehicle, or DNCB on days 92, 93, and 94, cyclophosphamide on days 95 and 96, and assayed on day 97.

On days 1-3 each mouse was dosed with 25 µl of DNCB or vehicle material on the dorsum of each ear for 3 consecutive days, allowing 24 +/- 2 hours between applications. Days 4-5 were days of rest.

Mice were weighed and then transported to the laboratory for the remainder of the assay on day 6. ^{125} IUDR was injected into mice i.v. in the lateral tail vein. Mice were sacrificed 5 hours later by CO_2 asphyxiation, and the auricular lymph nodes collected in a tissue culture tube containing HBBS. All draining lymph nodes collected from an individual animal were pooled. The lymph nodes were macerated to yield single cell suspensions.

After completion of the PBS wash, the supernatant was decanted and the pellet loosened by the addition of 5% trichloracetic acid (TCA). The tubes were vortexed and then refrigerated (2-8°C) overnight (16-24 hours). While incubating overnight, the tubes were placed in plastic beakers that were completely wrapped in lead foil. The single cell suspensions were removed from the refrigerator, vortexed, centrifuged and prepared for counting in the gamma counter.

Food and Water: Mice were provided with Purina 5001 Rodent Chow with access to tap water *ad libitum*. Water bottles were weighed daily to determine intake.

Environmental Control: Animals were maintained on 12 hour day and 12 hour night cycles.

Hormone Analysis

Sample Collection: Serum samples from control and perchlorate treated mice were received from BRT, Inc, RTP, NC. Ammonium perchlorate was administered orally in drinking water to groups of mice for periods of 14 or 90-days. The target doses were 0 (control), 0.02, 0.06, 0.2, and 2 mg/kg/day. The mice exposed for 14-days are shown in Table 1. The mice exposed for 90-days are shown in Table 2 (see Appendix B). Serum samples were collected after 14 or 90-days. Samples were kept frozen at -80^0 C prior to analysis for serum thyroid hormones. Additional mice were exposed to perchlorate for 14 days. The target dose was 0 (control) and 50 mg/kg/day.

Hormone Analysis: The following serum thyroid hormone levels were determined in control and perchlorate exposed female mice: thyroxine (T_4) and TSH. There was insufficient blood to measure T_3. Assays for T_4, and TSH were performed using radioimmunoassay (RIA) kits according to manufacturer's standard procedures and standard procedures for this laboratory (Narayanan and Mattie, 1998). Standards were run in triplicate while samples from the mice were run as individual samples due to the limited amount of blood available from a mouse. Blood from both the 14-day and 90-day time points were analyzed at the same time using assay kits from the same batch number and with the same expiration date for both T_4 or TSH measurements. Tracer (^{125}I) radioactivity was measured with a gamma counter (Packard Instrument Co., Meriden, CT). Sources of the RIA kits and antiserum/antibody were as follows: 1) T_4 RIA assay kits were purchased from Diagnostic Product Corp. (Los Angeles, CA) and T_4 antibody coated tubes were used; and 2) TSH RIA assay kits were purchased from Amersham Corp. (Arlington Heights, IL) and lyophilized rabbit anti-rat TSH serum and Amerlex-M second antibody (donkey anti-rabbit serum coated onto magnetized polymer particles containing sodium azide) were both used. Mice from the 14 day exposure to 0 or 50 mg/kg/day were analyzed using RIA kits with different lot numbers than the above kits used for the lower doses. Standards were run in triplicate while samples from the mice were run as individual samples due to the limited amount of blood available from a mouse.

Histopathology and S-Phase Labeling of Thyroids

All thyroids were processed by routine methods to paraffin as standard cross sections through both thyroids, trachea and esophagus. Paraffin blocks were sectioned at 5 microns and stained with either hematoxylin and eosin or anti-BrdU immunohistochemistry by routine methods. The thyroids were examined microscopically using the criteria previously developed while reviewing the series of perchlorate studies performed under Department of Defense contracts. The criteria were as follows:

Colloid depletion: Colloid depletion was considered present based on reduction or absence of colloid as evidenced by lack of eosinophilic protein in the follicular lumen or

pale, lacy and/or granular material in the follicular lumen. Loss of colloid is considered a more sensitive indication of response to treatment induced TSH increases than hypertrophy or hyperplasia.

Follicular cell hypertrophy: Follicular cell hypertrophy was considered present when thyroid follicles were uniformly lined by tall cuboidal to columnar epithelium. The cytoplasm was typically more basophilic than nonhypertrophic cells and had a lacy, sometimes vacuolated, appearance. There was an increase in cytoplasm to nuclear ratio along with an increased cell width and height

Hyperplasia

Hyperplasia was graded as described below:

0 - Follicles lined by normal appearing, squamous to short cuboidal epithelium with eosinophilic cytoplasm and normochromic nuclei.

1- Scattered individual or sometimes two adjacent follicles that have focal hyperplasia within the follicle. The areas of focal hyperplasia within a follicle were characterized by multiple layers of follicular epithelium usually 2-3 cells thick, protruding into the lumen of the follicle. There had to be 2 or more hyperplastic follicles, were required and follicles on the peripheral rim of the thyroid gland section were not counted.

2 - A greater number of scattered individual affected follicles or foci of more than 2 hyperplastic follicles. The areas of focal hyperplasia within a follicle were characterized by multiple layers of cuboidal follicular epithelium, usually more than 3 layers, protruding into the follicular lumen. These areas of hyperplasia could also have microfollicular formation within them.

DATA & STATISTICAL ANALYSIS

Plaque-Forming Cell Assay (PFC):

The mean of the duplicate plate counts for each dilution was calculated. The mean plaque count was multiplied by the dilution to obtain PFC/0.1 ml and multiplied by 10 to obtain PFC/ml. PFC/spleen were obtained by multiplying PFC/ml by 5 ml/spleen. PFC/10^6 spleen cells was obtained by dividing PFC/ml by the cell/ml expressed as (X) x10^6 cells/ml. If both sets of dilutions resulted in countable plaque numbers, then the average of the two values was calculated, so that only single mean value was obtained per animal. Means, standard deviations, and standard error of the mean (SE) values were calculated for each treatment group. Unacceptable plates were not included in calculations.

The PFC/10^6 spleen cells (Cells) and the PFC/Spleen (Spleen) counts were compared against vehicle by first performing a Bartlett's Chi-Square test for variance homogeneity. If this was found to be non-significant, a one-way analysis of variance was used using dose (concentration). If this was found to be significant, then a Dunnett's t test was performed using an alpha of 0.05.

If the Barlett's Chi-Square was found to be significant, non-parametric analyses were performed. Specifically, a Kruskal-Wallis test was performed. If this was found to be significant, then a Jonckheere's-Terpera test was performed for dose-dependent trends.

The 90 day experiments (lower doses: 0.02, 0.06, 0.2, and 2 mg, and high dose: 50 mg) were analyzed to evaluate the possibility of pooling the data from the two experiments. The AP-0 (vehicle) and AP-CP (vehicle + cyclophosphamide) controls were evaluated for differences between experiments. The poolability of groups of the low dose study and the high dose study was performed two ways: (1) the parametric ANOVA and (2) the non-parametric extended Cochran-Mantel-Haenszel Test. Both were not significant and the data were pooled prior to further statistical analysis.

No statistical analysis was performed on any of the pre-study optimization experiments.

LLNA:

The results from each cell suspension counted on the gamma counter were recorded in counts per minute (CPM). The CPMs were converted to disintegrations per minute (DPMs) by dividing by the gamma counter efficiency and multiplying by 100. After the DPM values were calculated, the mean "blank "DPM was subtracted from each mouse DPM to obtain corrected DPM values. The mean corrected DPM and standard error of the mean (SE) was determined for each treatment group. The stimulation index (SI) was calculated by dividing the treated group mean DPM by the control (vehicle) group mean DPM.

To test that DNCB was performing appropriately as a sensitizer, a one-sample t test was performed to confirm that the individual untransformed SI values of the DNCB control were different from 3.0. A sensitizer is defined by an SI value greater than or equal to three (3).

The natural log transformed dpm values for each treatment group was then compared against vehicle (AP-0 , vehicle control) by first performing a Bartlett's Chi-Square test for variance homogeneity. If this was found to be non-significant, a one-way analysis of variance was used using dose (concentration. If this was found to be significant, then a Dunnett's t test was performed using an alpha of 0.05.

If the Barlett's Chi-Square was found to be significant, non-parametric analyses were performed. Specifically, a Kruskal-Wallis test was performed. If this was found to be significant, then a Jonckheere's-Terpera test was performed for dose-dependent trends.

The 90 day experiments (lower doses: 0.02, 0.06, 0.2, and 2 mg, and high dose: 50 mg) were analyzed to evaluate the possibility of pooling the data from the two experiments. The AP-0 (DNCB control), and AP-0 (DNCB + cyclophosphamide), and AP-0 (vehicle control) controls were evaluated for differences between experiments. The poolability of groups of the low dose study and the high dose study was performed two ways: (1) the parametric ANOVA and (2) the non-parametric extended Cochran-Mantel-Haenszel Test. Both were not significant and the data were pooled prior to further statistical analysis.

No statistical analysis was performed on any of the pre-study optimization experiments.

Hormone Analysis:

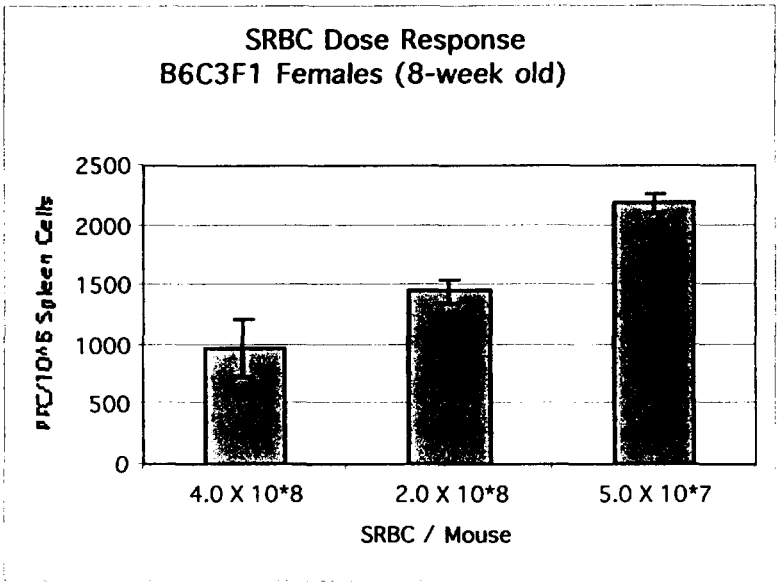
T_4 and TSH were analyzed from perchlorate exposed and control mice for both 14 and 90-day exposure periods: control, 0.02, 0.06, 0.2, and 2 mg/kg/day. Each combination of exposure period and dose group used different mice for T_4 and TSH since there was not enough serum to obtain both from the same mouse. A one-factor (dose group) analysis of variance was performed separately for each exposure period. Paired comparisons among the dose groups used 2-tailed t-tests with pooled error.

For the 50 mg/kg/day dose paired comparisons were used for the control and exposed groups for TSH and T_4. For T_4 there was a difference between the two 50 mg/kg/day groups so a 2-factor analysis of variance was performed with two factors, study number (studies: 110 & 100, studies: 906 & 901) and dose (control, 50mg/kg/day).

RESULTS

Sheep Red Blood Cell (SRBC) Titration for use in the PFC Assay:

Different numbers of sheep red blood cells (SRBC) were evaluated for use in the PFC assay using B6C3F1 female mice 8 weeks of age (Figure 1) and 19 weeks of age (Figure 2). The optimum SRBC number for immunization of mice at both 8 and 19



weeks of age in the PFC is 2.0 x 10^8 SRBC / mouse.

Figure 1 *: SRBC Titration – 8 week- old mice*

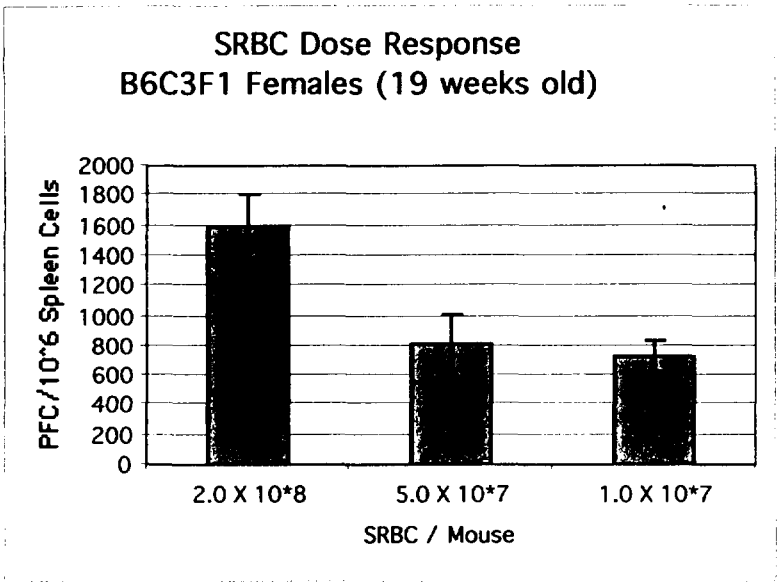
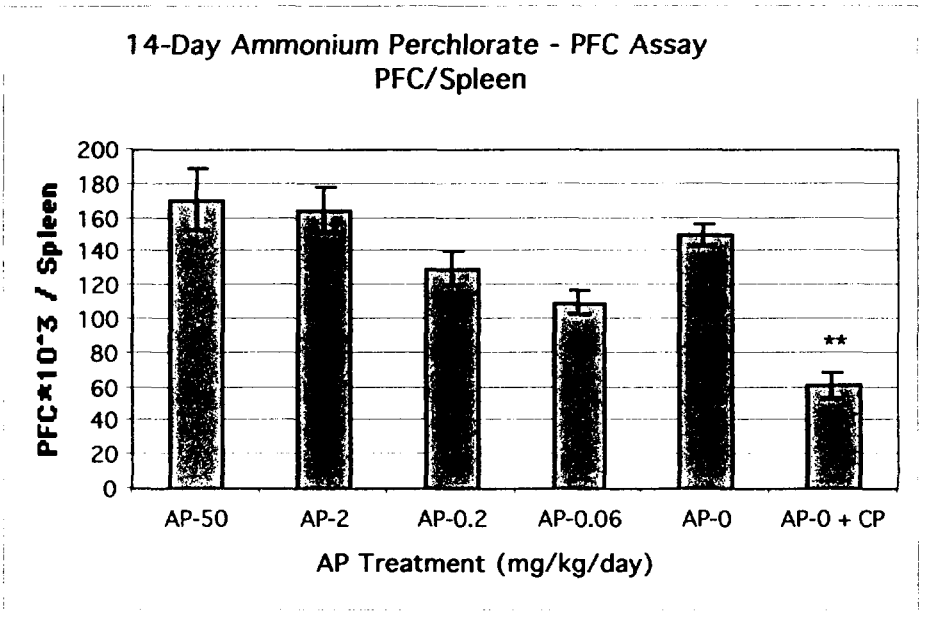


Figure 2 : *SRBC Titration – 19 week- old mice*

PFC-14 Days:

There was no statistically significant effect of ammonium perchlorate (AP) on the plaque forming cell (PFC) response in the 14 day study at any of the AP doses tested . There was no effect on the PFC per spleen (Figure 3) or PFC per 10^6 spleen cells at doses of 0.06, 0.20, 2.00 or 50.00 mg/kg/day (Figure 4). Injection of cyclophosphamide at 15 mg/kg intraperitoneally for 4 consecutive days prior to assay significantly ($p < 0.05$) suppressed the number of PFCs per spleen and the number of PFCs per 10^6 spleen cells.



Figures 3 : *14 Day Ammonium Perchlorate Study – Plaque Forming Cell Assay. PFC/Spleen.*

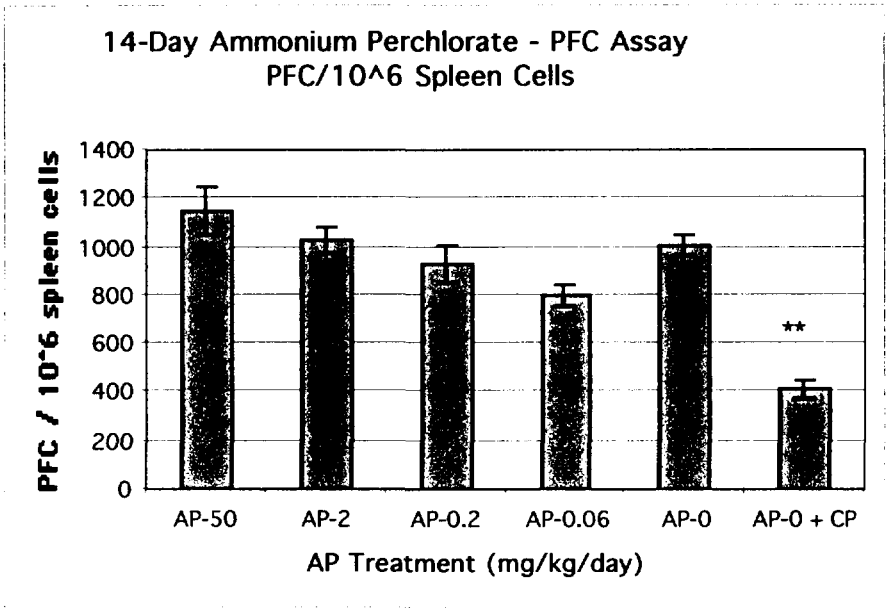


Figure 4: *14 Day Ammonium Perchlorate Study – Plaque Forming Cell Assay. PFC/10^6 Spleen Cells*

PFC-90 Days:

In the 90 day study, ammonium perchlorate had no effect on PFC at 0.02, 0.06 or 0.20 mg/kg/day. However, a statistically significant increase ($p < 0.05$) in the number of PFCs per spleen occurred at doses of 2.00 and 50.00 mg/kg/day, while there was an increase in the number of PFCs per 10^6 spleen cells at 50.00 mg/kg/day. Injection of cyclophosphamide at 15 mg/kg intraperitoneally for 4 consecutive days prior to assay significantly ($p < 0.05$) suppressed the number of PFCs per spleen and the number of PFCs per 10^6 spleen cells in the 90 day assay.

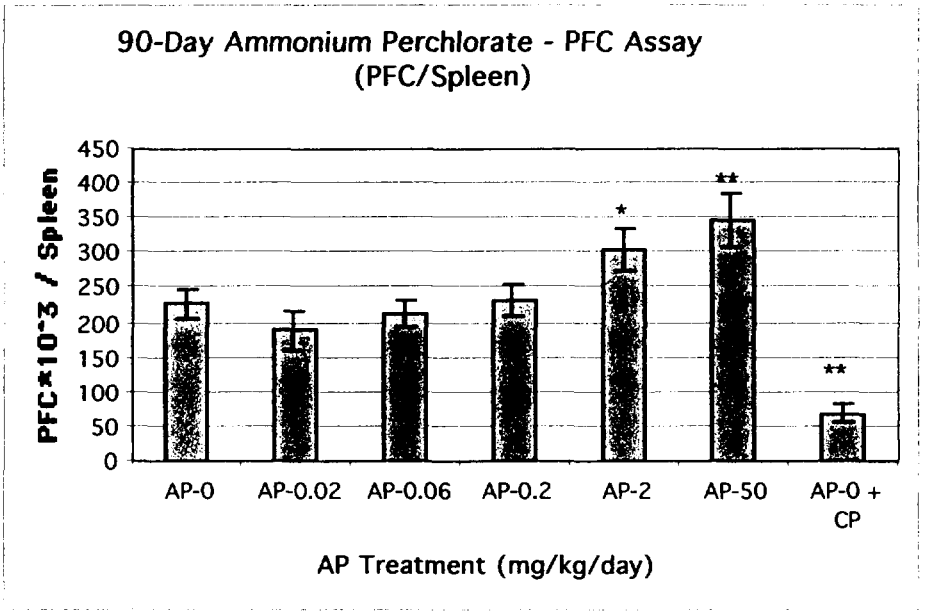


Figure 5: *90 Day Ammonium Perchlorate Study – Plaque Forming Cell Assay. PFC/Spleen*

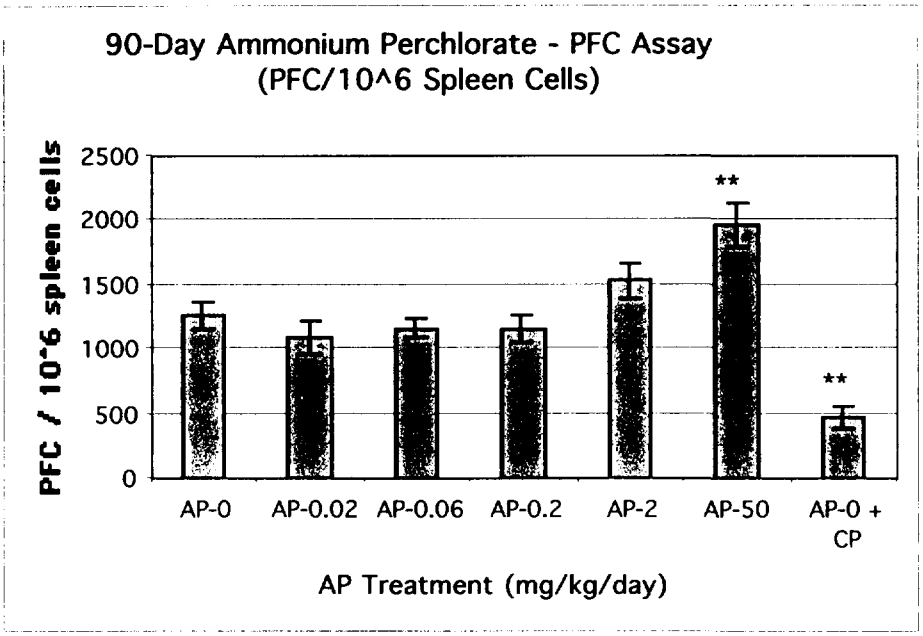


Figure 6: *90 Day Ammonium Perchlorate Study – Plaque Forming Cell Assay. PFC/10^6 Spleen Cells*

DNCB Titration:

DNCB was titrated to determine the optimum dose for use in subsequent assays. Doses of 0.1%, 0.25%, and 0.5% DNCB were tested and all increased lymphocyte proliferation (Figure 5). A dose of 0.25% DNCB was chosen for use in the LLNA to evaluate the effect of ammonium perchlorate on contact sensitization induced by DNCB.

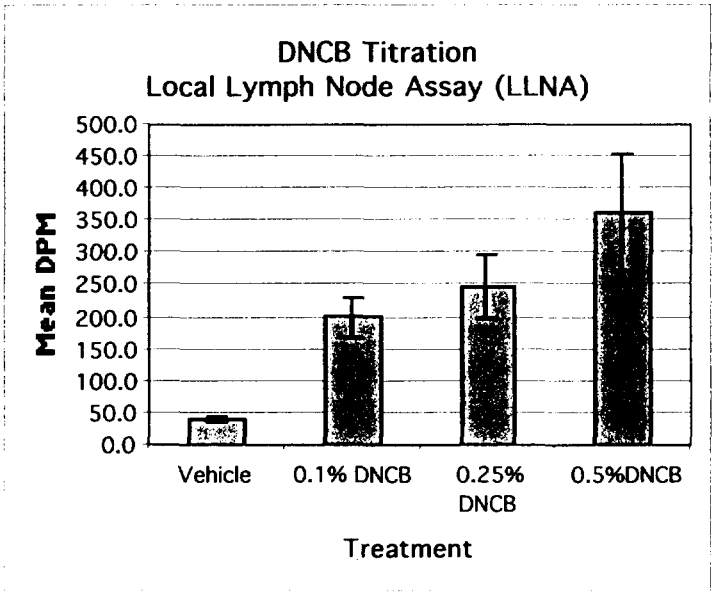


Figure 7: *DNCB Titration for LLNA*

LLNA-14 Days:

DNCB was a sensitizer as demonstrated by a stimulation index (SI) statistically greater than 3 (one-sample t-test with $p < 0.05$). Ammonium perchlorate (AP) enhanced the sensitization by DNCB at doses of 0.06, 0.2 and 50 mg/kg/day. Cyclophosphamide (AP-0/DNCB/CP) suppressed the contact sensitization induced by DNCB (AP-0/DNCB, $p=0.0017$ unpaired t Test).

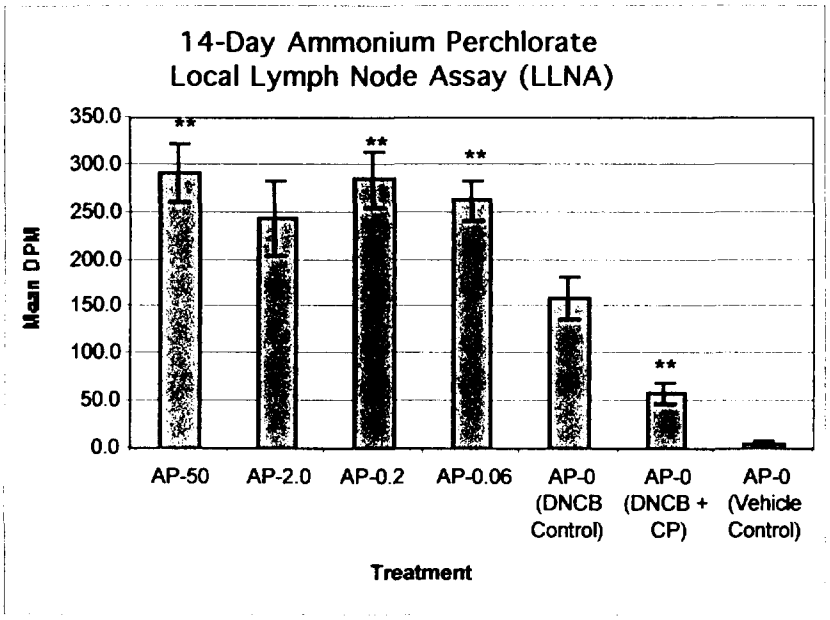


Figure 8: *14 Day Ammonium Perchlorate Study – Local Lymph Node Assay*

LLNA-90 Days:

DNCB was a sensitizer as demonstrated by a stimulation index (SI) statistically greater than 3 (one-sample t-test with $p < 0.05$). Ammonium perchlorate (AP) enhanced the contact sensitivity response to DNCB at doses of 0.06 and 0.2 and suppressed the response at 50 mg/kg/day (Dunnett's Test as a post-hoc test, $p=0.05$). Cyclophosphamide (CP) in the 90 day study did not significantly suppress the contact sensitization induced by DCNB.

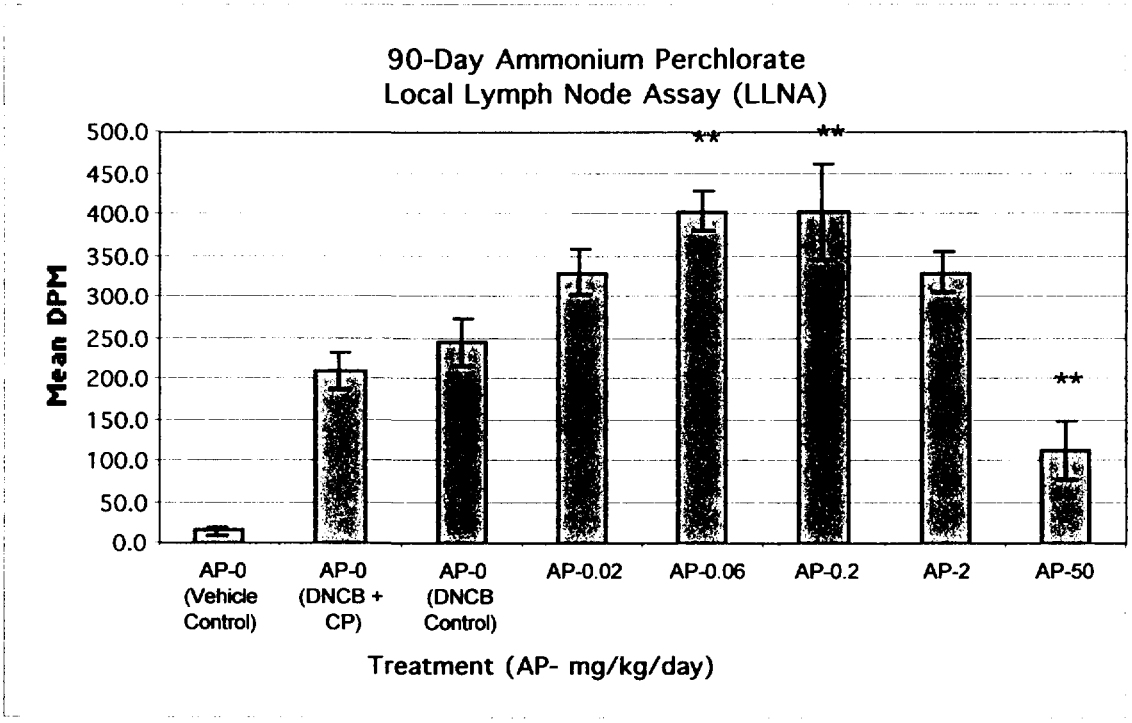


Figure 9: *90 Day Ammonium Perchlorate Study – Local Lymph Node Assay*

T_4 and TSH-14 Days:

After 14 days of exposure to perchlorate by B6C3F1 female mice the no effect level for T_4 in sera was 0.06 mg/kg/day. A significant decrease of T_4 in sera was observed at 0.20 ($p=0.0166$) and 2.00 mg/kg/day($p=0.0073$). Although TSH levels after 14 days of dosing were starting to increase with increasing dose, a significant increase was not seen until the 2 mg/kg/day dose. At the 50 mg/kg/day dose, both T_4 and TSH levels were significantly different from control levels at $p=0.0001$. See Appendix B for a more detailed report, figures and tables for hormone results.

T_4 and TSH-90 Days

After 90 days of exposure to perchlorate, T_4 levels in sera were not significantly lower until the high dose group. The 0.2 mg/kg/day dose resulted in lower T_4 values but the range of data was great enough that the decrease was not significant. After 90 days of exposure to perchlorate in drinking water, TSH levels were elevated at a much lower dose (0.06 mg/kg/day). See Appendix B for a more detailed report, figures and tables for hormone results. Significant changes were not seen for either hormone until the percent change from control exceeded 12%.

Thyroid alterations:

3/15 mice treated with 2 mg/kg/day ammonium perchlorate for 90 days had hypertrophy. 5/5 mice treated with 50 mg/kg/day for 14 days had both colloid depletion and hypertrophy. None of the mice from any other exposure group were different from their concurrent control. S-phase labeling was not different from control in any treatment group. The slides from mice treated with 50 mg/kg/day for 90 days and their concurrent control have not been examined as the tissues are still being processed as of 6/23/2000.

DISCUSSION

There was no effect of ammonium perchlorate on the plaque-forming cell (PFC) response in the 14 day study. Similarly, there was no effect on the PFC per spleen or PFC per 10^6 spleen cells at doses of 0.06, 0.20, 2.00 or 50.00 mg/kg/day. Cyclophosphamide at 15 mg/kg intraperitoneally 4 days prior to assay significantly ($p < 0.05$) suppressed the number of PFCs per spleen and the number of PFCs per 10^6 spleen cells. In the 90 day study, ammonium perchlorate had no effect on PFC at 0.02, 0.06 or 0.20 mg/kg/day. However, a statistically significant increase ($p < 0.05$) in the number of PFCs per spleen occurred at doses of 2.00 and 50.00 mg/kg/day, while there was an increase in the number of PFCs per 10^6 spleen cells at 50.00 mg/kg/day. Cyclophosphamide at 15 mg/kg intraperitoneally 4 days prior to assay significantly ($p < 0.05$) suppressed the number of PFCs per spleen and the number of PFCs per 10^6 spleen cells in the 90 day study. Despite the many studies of neuroendocrine-immune system interactions, the relationship between the thyroid axis and immunological function is not clearly established. Klecha et al (2000) reported a study demonstrating that treatment with exogenous T_4 results in increased alloantibody titers. These authors also demonstrated that propylthiouracil treatment to reduce T_4 levels decreased both the humoral and the cellular immune response. The present study demonstrated decreased levels of T_4 as well as increased TSH levels without a concomitant decrease, and indeed an increase, in humoral immunity. This lack of correlation may be due to timing of ammonium perchlorate relative to antigen stimulation, dose of ammonium perchlorate, the level of hormone suppression (T_4) or stimulation (TSH), the ratio of T_4:TSH, or the possibility that T_4 and/or TSH levels are not related to humoral immunity. After 14 days of exposure to perchlorate in B6C3F1 female mice, the noeffect level for T_4 in sera was 0.06 mg/kg/day. Although TSH levels after 14 days of dosing were starting to increase with increasing dose, a significant increase was not seen until the 2 mg/kg/day dose. At the 50 mg/kg/day dose, both T_4 and TSH levels were significantly different from control levels ($p=0.0001$). After 90 days of exposure to perchlorate, T_4 levels in sera were not significantly lower except in the high dose group. The 0.2 mg/kg/day dose resulted in lower T_4 values but the range of data was great enough that the decrease was not significant. After 90 days of exposure to perchlorate in drinking water TSH levels were elevated at a much lower dose (0.06 mg/kg/day. The lack of suppression in the humoral immunity due to increased T_4 is likely due to the increased levels of TSH. TSH, released from the pituitary gland, is the major regulator of thyroid function and structure (Larsen and Ingbar, 1992). An increase in TSH levels in the blood results in an increase in the release of T_4 from the thyroid gland. Iodine deficiency leads to a decrease in T_4 production, an increase in thyroid releasing hormone (TRH) from the hypothalamus and an increase in TSH from the pituitary. The initial step in the production of T_4 is the uptake of iodine from plasma by thyroid follicular cells. Perchlorate is one of a number of anions that competitively inhibits iodine transport into the thyroid producing an effect similar to dietary iodine deficiency (Capen, 1997). This ability of perchlorate to inhibit iodide uptake was the basis for the medicinal use of perchlorate salts to control hyperthyroidism. Due to cases of agranulocytosis and aplastic anemia (with fatalities), perchlorates are no longer

used medicinally in the United States (Larsen and Ingbar, 1992). Although perchlorate salts were used pharmacologically, subchronic or chronic dose-response studies were not conducted to examine the long-term effects of perchlorate anion on the thyroid until recently.

The mechanism for the increase in PFC at 2.0 and 50.0 mg/kg/day ammonium perchlorate and the increased contact sensitization induced by DNCB at 0.06, 0.20, 2.00 or 50.00 mg/kg/day may find parallel in studies with cyclophosphamide (CP). Acute exposure of mice to CP one day prior to challenge with *Listeria monocytogenes* suppresses host resistance (Morahan et al. 1984; Tripathy and Mackaness, 1969). However, subchronic treatment of mice prior to challenge enhanced host resistance (Luster et al., 1981). Both acute and subchronic exposures to CP cause depletion of lymphocytes, but the number of granulocyte-macrophage progenitor cells were increased in the bone marrow of mice treated subchronically, when compared to untreated or acutely treated mice.

Although contact hypersensitivity has been considered to be a T-cell mediated event, there is an increase in the number of B-cells in the draining lymph nodes following sensitization. Delayed hypersensitivity reactions including the tuberculin type, Jones Mote type reactions, and contact hypersensitivity are increased by pre-treating with a single dose of CP (Turk, 1987). The contact sensitizer DNFB results in the proliferation of lymphocyte subsets in the draining auricular and in the draining and contralateral cervical lymph nodes. Baker et al (1987) reported that pretreatment with CP results in enhanced contact sensitivity and hypothesize the mechanism to be a decrease in B-suppressor cells and a decrease in T-suppressor/cytotoxic cells. Thus, CP is effective in both suppressing and in increasing contact skin reactions depending on the time and dose of administration (Parker and Turk, 1982). Likewise, the studies with AP can both suppress and enhance the immune response, depending on the particular exposure dose, exposure regimen, and the immunological function evaluated.

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APPENDIX A

Statistical Analysis

APPENDIX B

Hormone Analysis

AN IMMUNOTOXICITY STUDY OF AMMONIUM PERCHLORATE ADMINSITERED IN DRINKING WATER TO MICE:

SERUM HORMONE (TSH, T_3, o) REPORT

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Introduction

Ammonium perchlorate is an oxidizer that has been used as a component of solid rocket fuel. Perchlorate, the dissociated ion of ammonium perchlorate, has recently been recognized as a persistent and pervasive contaminant of water supplies in a number of major metropolitan areas. Current efforts at assessing the health risks of perchlorate have been hampered by a lack of relevant toxicity data and by a lack of understanding of the potential toxic mechanism of action. Perchlorate is known to disrupt thyroid hormone homeostasis in a number of species via an inhibition of iodine uptake into the thyroid gland. Assessment of thyroid hormone (T_3 and T_4) and thyroid stimulating hormone (TSH) levels in circulating blood is the most sensitive endpoint for the effect of perchlorate on an organism. Caldwell *et al.* (1996) measured T_3,T_4 and TSH hormone levels in male and female rats after 14 days of exposure to ammonium perchlorate in drinking water. The values for T_3 and T_4 in both male and female Sprague-Dawley rats decreased while the concentration of TSH increased after 14 days. Thyroid organ weights were not measured but histopathology of the thyroid revealed hypertrophy of follicular cells at the highest concentration levels. One objective of the Immunotoxicity Study was to examine thyroid hormone (T_4) and TSH levels to assess if changes were occurring in mice exposed to AP for 14 or 90-days.

Methods

Compliance Statement: The analyses in this report were conducted to the fullest extent possible according to the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792.

Sample Collection: Serum samples from control and perchlorate treated mice were received from BRT, Inc, RTP, NC. Ammonium perchlorate was administered orally in drinking water to groups of mice for periods of 14 or 90-days. The target doses were 0 (control), 0.02, 0.06, 0.2, and 2 mg/kg/day. The mice exposed for 14-days are shown in Table 1. The mice exposed for 90-days are shown in Table 2. Serum samples were collected after 14 or 90-days. Samples were kept frozen at -80^0 C prior to analysis for serum thyroid hormones. Additional mice were exposed to perchlorate for 14 days. The target dose was 0 (control) and 50 mg/kg/day.

Hormone Analysis: The following serum thyroid hormone levels were determined in control and perchlorate exposed female mice: thyroxine (T_4) and thyroid - stimulating hormone (TSH). There was insufficient blood to measure T_3. Assays for T_4, and TSH were performed using radioimmunoassay (RIA) kits according to manufacturer's standard procedures and standard procedures for this laboratory (Narayanan and Mattie, 1998). Standards were run in triplicate while samples from the mice were run as individual samples due to the limited amount of blood available from a mouse. Blood from both the 14-day and 90-day time points were analyzed at the same time using assay kits from the same batch number and with the same expiration date for both T_4 or TSH measurements. Tracer (^{125}I) radioactivity was measured with a gamma counter (Packard Instrument Co., Meriden, CT). Sources of the RIA kits and antiserum/antibody were: 1) T_4 RIA assay kits were purchased from Diagnostic Product Corp. (Los Angeles, CA) and T_4 antibody coated tubes were used; and 2) TSH RIA assay kits were purchased from Amersham Corp. (Arlington Heights, IL) and lyophilized rabbit anti-rat TSH serum and Amerlex-M second antibody (donkey anti-rabbit serum coated onto magnetized polymer particles containing sodium azide) were both used. Mice from the 14 day exposure to 0 or 50 mg/kg/day were analyzed using RIA kits with different lot numbers than the above kits used for the lower doses. Standards were run in triplicate while samples from the mice were run as individual samples due to the limited amount of blood available from a mouse.

Statistical Analysis: T_4 and TSH were analyzed from perchlorate exposed and control mice for both 14 and 90-day exposure periods: control, 0.02, 0.06, 0.2, and 2 mg/kg/day. Each combination of exposure period and dose group used different mice for T_4 and TSH since there was not enough serum to obtain both from the same mouse. A one-factor (dose group) analysis of variance was performed separately for each exposure period. Paired comparisons among the dose groups used 2-tailed t-tests with pooled error.

For the 50 mg/kg/day dose paired comparisons were used for the control and exposed groups for TSH and T_4. For T_4 there was a difference between the two 50 mg/kg/day groups so a 2-factor analysis of variance was performed with two factors, study number (studies: 110 & 100, studies: 906 & 901) and dose (control, 50mg/kg/day).

Results: The results for T_4 are shown in Figure 1. The spread of values for individual animals is shown as well as the means for each group. Paired comparisons of dose group for T_4 are found in Table 1. There was not a significant difference among the dose groups for T_4 at 14 days {$F(4,28)=2.59$, $p=0.0582$}. There was a significant difference among the dose groups for T_4 at 90 days {$F(4,29)=2.87$, $p=0.0404$}. The mean percent change from control for T_4 is show in Figure 2.

The results for TSH are shown in Figure 3. The spread of values for individual animals is shown as well as the means for each group. Paired comparisons of dose group for TSH are found in Table 2. TSH was significantly different at 14 days {$F(4,28)=14.02$, $p=0.0001$}, and at 90 days {$F(4,30)=4.09$, $p=0.0092$}. The mean percent change from control for TSH is show in Figure 4.

Table 3 contains results of relevant paired comparisons by study number for T_4. Note that there was a significant difference in the two 50mg/kg/day study groups ($p=0.0043$). All 5 T_4 values for the 901 study group were higher than all 5 values for the 100 study group.

Results of the 2-factor analysis of variance performed with two factors, study number (studies: 110 & 100, studies: 906 & 901) and dose (control, 50mg/kg/day) are shown in Table 4.

TSH values were missing for all of the 906 study group. For the 100 study group there was only an N=2. Since the 2 values of TSH in the 100 study group were within the range of TSH values in the 901 study group, the study difference was ignored and all 7 values were considered as the same 50 mg/kg/day group. Results of the one-factor (dose) analysis of variance for TSH are shown in Table 4.

Table 5 contains the dose means for the main effect for both T_4 and TSH.

Day	Dose Group mg/kg/day	N	Mean T_4 (ug/dL)	Std Dev T_4 (ug/dL)	Dose Group mg/kg/day 0.02	0.06	0.2	2
14	Control	7	7.08	0.92	0.0573	0.0533	0.0166	0.0073
	0.02	7	6.27	0.84		0.9723	0.4662	0.3711
	0.06	7	6.26	0.68			0.4856	0.3896
	0.2	5	5.94	0.52				0.9282
	2	7	5.90	0.71				
90	Control	8	6.05	0.78	0.7098	0.7370	0.2620	0.0167
	0.02	8	6.19	0.64		0.9929	0.1465	0.0073
	0.06	6	6.19	0.96			0.1759	0.0116
	0.2	6	5.57	0.92				0.2019
	2	6	4.98	0.58				

Table 1. Paired comparisons of dose group for T_4. Values listed under each dose column are p-values (2-tailed t-test with pooled error) for comparing row dose and column dose.

Day	Dose Group mg/kg/day	N	Mean TSH (ng/mL)	Std Dev TSH (ng/mL)	Dose Group mg/kg/day 0.02	0.06	0.2	2
14	Control	8	2.94	0.29	0.9359	0.2600	0.0741	0.0001
	0.02	6	2.93	0.32		0.2594	0.0808	0.0001
	0.06	6	3.14	0.26			0.5144	0.0001
	0.2	6	3.27	0.32				0.0002
	2	7	4.05	0.41				
90	Control	7	3.43	0.40	0.1720	0.0084	0.0031	0.0023
	0.02	7	3.72	0.42		0.1658	0.0800	0.0621
	0.06	7	4.01	0.33			0.6980	0.6084
	0.2	7	4.09	0.39				0.9006
	2	7	4.11	0.36				

Table 2. Paired comparisons of dose group for TSH. Values listed under each dose column are p-values (2-tailed t-test with pooled error) for comparing row dose and column dose.

Dose Group mg/kg/day	N	Mean T_4 (ug/dL)	Std Dev T_4 (ug/dL)	Dose Group mg/kg/day		
				906-ctrl	100-50mg	901-50mg
110-ctrl	5	5.09	0.50	0.7588	0.0001	
906-ctrl	5	5.01	0.15			0.0001
100-50mg	5	2.70	0.19			0.0043
901-50mg	5	3.23	0.23			

Table 3. Relevant paired comparisons of dose group for T_4. Values listed under each dose column are p-values (2-tailed t-test) for comparing row dose and column dose.

Hormone	Source	DF	SS	F	P
T_4	study no.	1	2.55E-01	2.82	0.1127
	dose	1	2.17E+01	239.12	0.0001
	study no. x dose	1	4.56E-01	5.03	0.0394
	error	16	1.45E+00		
	total	19	2.38E+01		
TSH	dose	1	2.07E+00	58.03	0.0001
	error	10	3.56E-01		
	total	11	2.42E+00		

Table 4. Analysis of variance results with T_4 and TSH hormone levels as the dependent variable.

Dose	N T_4	Mean T_4 ug/dL	Std T_4 ug/dL	N TSH	Mean TSH ng/mL	Std TSH ng/mL
Control	10	5.05	0.37	5	2.37	0.11
50 mg	10	2.97	0.21	7	3.22	0.23

Table 5. Main effect means for dose. For T_4, the standard deviation is pooled across study groups.

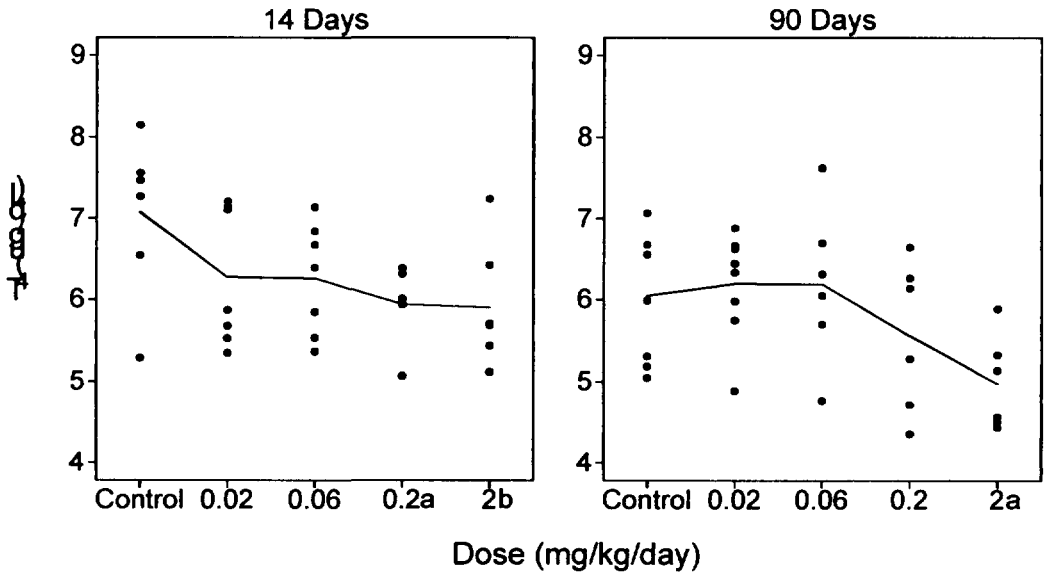

Figure 1. T_4 for each mouse. Line segments connect means from each dose group. Comparisons with control (a: $0.01<p\leq0.05$, b: $0.001<p\leq0.01$, c: $p\leq0.001$) used 2-tailed t-tests with pooled error.

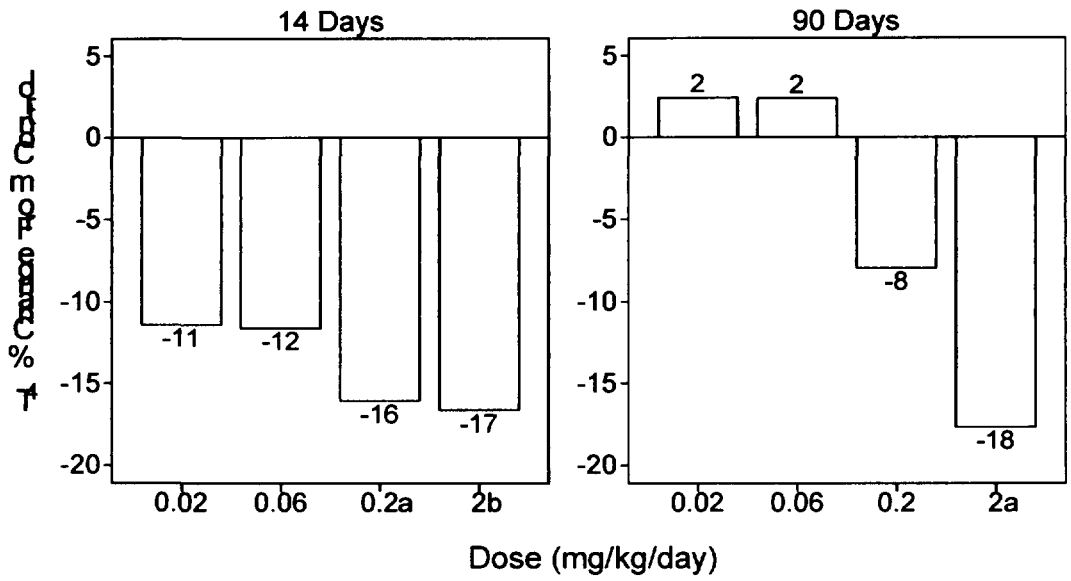

Figure 2. Mean percent change from control for T_4. Comparisons with control (a: $0.01<p\leq0.05$, b: $0.001<p\leq0.01$, c: $p\leq0.001$) used 2-tailed t-tests with pooled error.

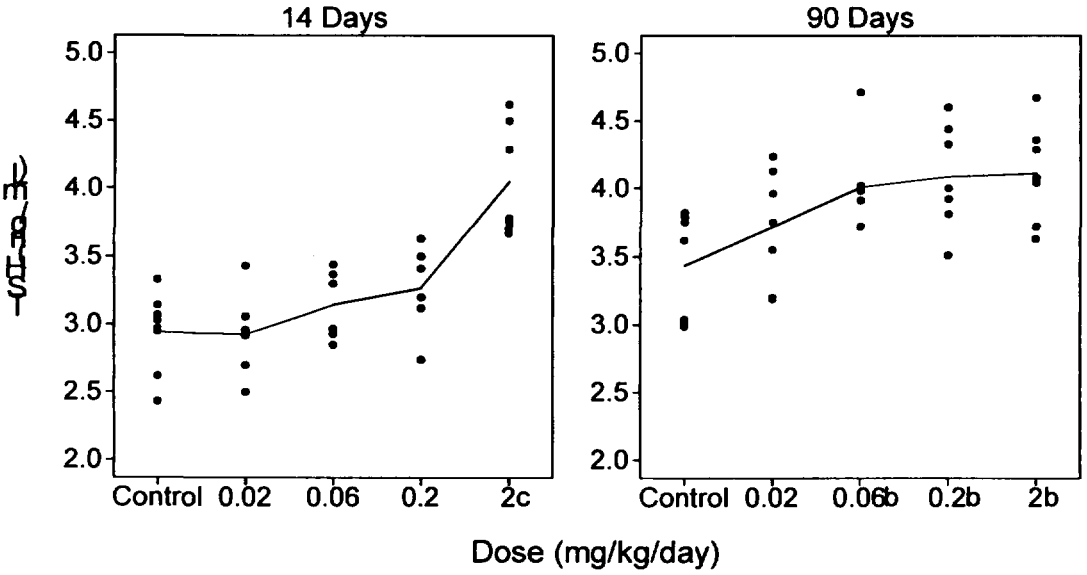

Figure 3. TSH for each mouse. Line segments connect means from each dose group. Comparisons with control (a: $0.01<p\leq0.05$, b: $0.001<p\leq0.01$, c: $p\leq0.001$) used 2-tailed t-tests with pooled error.

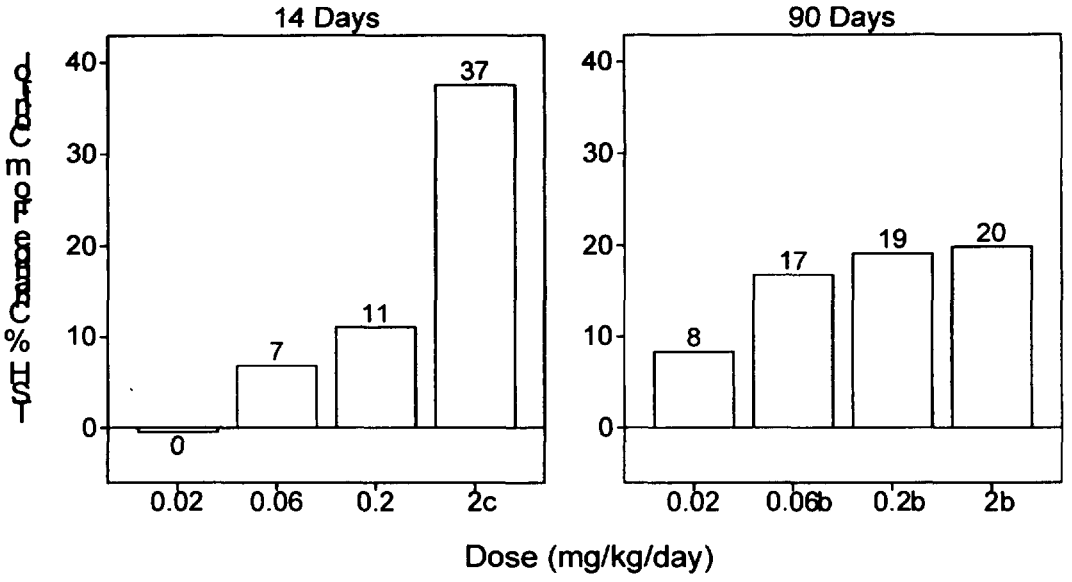

Figure 4. Mean percent change from control for TSH. Comparisons with control (a: $0.01<p\leq0.05$, b: $0.001<p\leq0.01$, c: $p\leq0.001$) used 2-tailed t-tests with pooled error.

Discussion: TSH, released from the pituitary gland, is the major regulator of thyroid function and structure (Larsen and Ingbar, 1992). An increase in TSH levels in the blood results in an increase in the release of T_4 from the thyroid gland. Iodine deficiency leads to a decrease in T_4 production, an increase in thyroid releasing hormone (TRH) from the hypothalamus and an increase in TSH from the pituitary. The initial step in the production of T_4 is the uptake of iodine from plasma by thyroid follicular cells. Perchlorate is one of a number of anions that competitively inhibits iodine transport into the thyroid producing an effect similar to dietary iodine deficiency (Capen, 1997). This ability of perchlorate to inhibit iodide uptake was the basis for the medicinal use of perchlorate salts to control hyperthyroidism. Due to cases of agranulocytosis and aplastic anemia (with fatalities), perchlorates are no longer used medicinally in the United States (Larsen and Ingbar, 1992). Although perchlorate salts were used pharmacologically, subchronic or chronic dose-response studies were not conducted to examine the long-term effects of perchlorate anion on the thyroid until recently. Siglin *et al.* (1999) examined rats after 90-days of exposure to perchlorate in drinking water in order to determine target organs and effect levels. Kiel *et al.* (1999) exposed mice for 90-days to perchlorate in drinking water in order to examine certain immunological endpoints.

After 14 days of exposure to perchlorate by B6C3F1 female mice the no effect level for T_4 in sera was 0.06 mg/kg/day. Although TSH levels after 14 days of dosing were starting to increase with increasing dose, a significant increase was not seen until the 2 mg/kg/day dose. At the 50 mg/kg/day dose, both T_4 and TSH levels were significantly different from control levels at $p=0.0001$.

After 90 days of exposure to perchlorate, T_4 levels in sera were not significantly lower until the high dose group. The 0.2 mg/kg/day dose resulted in lower T_4 values but the range of data was great

enough that the decrease was not significant. After 90 days of exposure to perchlorate in drinking water TSH levels were elevated at a much lower dose (0.06 mg/kg/day). Significant changes were not seen for either hormone until the percent change from control exceeded 12%.

Acknowledgement: Special thanks go to Chuck Goodyear for performing statistical analyses of this hormone data. Mr. Goodyear is a statistical consultant for AFRL, Human Effectiveness Directorate, Crew Systems Interface Division (AFRL/HEC).

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APPENDIX C

Histopathology and s-phase labeling of thyroids from mice treated with ammonium perchlorate for 14 or 90 days.

Data collected and reported by Douglas C. Wolf, D.V.M., Ph.D., Environmental Carcinogenesis Division NHEERL/ORD/USEPA.

All thyroids were processed by routine methods to paraffin as standard cross sections through both thyroids, trachea and esophagus. Paraffin blocks were sectioned at 5 microns and stained with either hematoxylin and eosin or anti-BrdU immunohistochemistry by routine methods.
The thyroids were examined microscopically using the criteria previously developed while reviewing the series of perchlorate studies performed under Department of Defense contracts. The criteria follow.

Colloid depletion

Colloid depletion was considered present based on reduction or absence of colloid as evidenced by lack of eosinophilic protein in the follicular lumen or pale, lacy and/or granular material in the follicular lumen. Loss of colloid is considered a more sensitive indication of response to treatment induced TSH increases than hypertrophy or hyperplasia.

Follicular cell hypertrophy

Follicular cell hypertrophy was considered present when thyroid follicles were uniformly lined by tall cuboidal to columnar epithelium. The cytoplasm was typically more basophilic than nonhypertrophic cells and had a lacy, sometimes vacuolated, appearance. There was an increase in cytoplasm to nuclear ratio along with an increased cell width and height
Hyperplasia

Hyperplasia was graded as:

0 - follicles lined by normal appearing, squamous to short cuboidal epithelium with eosinophilic cytoplasm and normochromic nuclei.

1- scattered individual or sometimes two adjacent follicles that have focal hyperplasia within the follicle. The areas of focal hyperplasia within a follicle were characterized by multiple layers of follicular epithelium usually 2-3 cells thick protruding into the lumen of the follicle. There had to be 2 or more hyperplastic follicles and follicles on the peripheral rim of the thyroid gland section were not counted.

2 - a greater number of scattered individual affected follicles or foci of more than 2 hyperplastic follicles. The areas of focal hyperplasia within a follicle were characterized by multiple layers of cuboidal follicular epithelium, usually more than 3 layers, protruding into the follicular lumen. These areas of hyperplasia could also have microfollicular formation within them.

Thyroid alterations:

3/15 mice treated with 2 mg/kg/day ammonium perchlorate for 90 days had hypertrophy. 5/5 mice treated with 50 mg/kg/day for 14 days had both colloid depletion and hypertrophy. None of the mice from any other exposure group were different from their concurrent control. S-phase labeling was not different from control in any treatment group.

The slides from mice treated with 50 mg/kg/day for 90 days and their concurrent control have not been examined as the tissues are still being processed as of 6/23/2000.

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	2	Vehicle + CP Vehicle Control

Number of observations in data set = 20

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dependent Variable: CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1766224.05244038	1766224.05244038	55.08	0.0001
Error	18	577238.57251289	32068.80958405		
Corrected Total	19	2343462.62495328			

R-Square	C.V.	Root MSE	CELLS Mean
0.753681	25.19086	179.07766355	710.88341029

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	1766224.05244038	1766224.05244038	55.08	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	1766224.05244038	1766224.05244038	55.08	0.0001

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dependent Variable: SPLEEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	40028.87812500	40028.87812500	37.94	0.0001
Error	18	18992.76250000	1055.15347222		
Corrected Total	19	59021.64062500			

R-Square	C.V.	Root MSE	SPLEEN Mean
0.678207	30.77146	32.48312596	105.56250000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	40028.87812500	40028.87812500	37.94	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	40028.87812500	40028.87812500	37.94	0.0001

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Bartlett's Test for Equality of CELLS Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	1	0.2930	0.5883

Bartlett's Test for Equality of SPLEEN Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	1	0.8583	0.3542

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dunnett's T tests for variable: CELLS

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 32068.81
Critical Value of Dunnett's T= 2.101
Minimum Significant Difference= 168.26

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
Vehicle + CP	- Vehicle Control	-762.60	-594.34	-426.08	***

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dunnett's T tests for variable: SPLEEN

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 1055.153
Critical Value of Dunnett's T= 2.101
Minimum Significant Difference= 30.521

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
Vehicle + CP	- Vehicle Control	-120.00	-89.48	-58.95	***

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	5	AP-0.06 mg/kg/da AP-0.2 mg/kg/day AP-2.0 mg/kg/day AP-50 mg/kg/day Vehicle Control

Number of observations in data set = 50

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dependent Variable: CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	676622.22625987	169155.55656497	1.91	0.1256
Error	45	3990294.92093722	88673.22046527		
Corrected Total	49	4666917.14719709			

R-Square	C.V.	Root MSE	CELLS Mean
0.144983	30.33022	297.78049040	981.79484514

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	4	676622.22625987	169155.55656497	1.91	0.1256

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	4	676622.22625987	169155.55656497	1.91	0.1256

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dependent Variable: SPLEEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	25174.15750000	6293.53937500	2.29	0.0743
Error	45	123661.43750000	2748.03194444		
Corrected Total	49	148835.59500000			

R-Square	C.V.	Root MSE	SPLEEN Mean
0.169141	36.11303	52.42167438	145.16000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	4	25174.15750000	6293.53937500	2.29	0.0743

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	4	25174.15750000	6293.53937500	2.29	0.0743

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Bartlett's Test for Equality of CELLS Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	4	8.9565	0.0622

Bartlett's Test for Equality of SPLEEN Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	4	14.7493	0.0053

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dunnett's T tests for variable: CELLS

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 45 MSE= 88673.22
Critical Value of Dunnett's T= 2.531
Minimum Significant Difference= 337.1

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
AP-50 mg/kg/day - Vehicle Control	-194.4	142.7	479.8
AP-2.0 mg/kg/day - Vehicle Control	-321.7	15.4	352.5
AP-0.2 mg/kg/day - Vehicle Control	-416.2	-79.1	258.0
AP-0.06 mg/kg/da - Vehicle Control	-547.4	-210.3	126.7

One-Way ANOVA on EPA / Air Force 14 Day Plaque-Forming Cell (PFC) Assay in Mice

General Linear Models Procedure

Dunnett's T tests for variable: SPLEEN

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 45 MSE= 2748.032
Critical Value of Dunnett's T= 2.531
Minimum Significant Difference= 59.343

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
AP-50 mg/kg/day - Vehicle Control	-39.19	20.15	79.49
AP-2.0 mg/kg/day - Vehicle Control	-44.84	14.50	73.84
AP-0.2 mg/kg/day - Vehicle Control	-79.22	-19.88	39.47
AP-0.06 mg/kg/da - Vehicle Control	-99.82	-40.48	18.87

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40	CCOUNT7	CCOUNT6	VIABIL	PFC6_A
1	8-1	AP-0.06 mg/kg/day	149	124	54	.	3.166	31.66	99	862.287
2	8-2	AP-0.06 mg/kg/day	83	87	38	42	2.31	23.1	97	735.931
3	8-3	AP-0.06 mg/kg/day	123	95	48	49	3.072	30.72	98	709.635
4	8-4	AP-0.06 mg/kg/day	102	96	51	42	2.622	26.22	99	755.149
5	8-5	AP-0.06 mg/kg/day	97	72	48	31	3.186	31.86	96	530.446
6	9-1	AP-0.06 mg/kg/day	161	180	98	86	2.717	27.17	98	1255.06
7	9-2	AP-0.06 mg/kg/day	88	91	70	46	2.804	28.04	100	638.374
8	9-3	AP-0.06 mg/kg/day	111	93	46	60	2.497	24.97	98	816.98
9	9-4	AP-0.06 mg/kg/day	85	65	54	52	2.38	23.8	100	630.252
10	9-5	AP-0.06 mg/kg/day	126	135	80	66	2.931	29.31	100	890.481
11	6-1	AP-0.2 mg/kg/day	137	148	84	67	2.829	28.29	99	1007.42
12	6-2	AP-0.2 mg/kg/day	80	78	69	89	3.381	33.81	99	467.317
13	6-3	AP-0.2 mg/kg/day	.	97	89	62	2.933	29.33	100	661.439
14	6-4	AP-0.2 mg/kg/day	262	241	98	85	3.109	31.09	100	1617.88
15	6-5	AP-0.2 mg/kg/day	202	162	104	93	2.55	25.5	100	1427.45

OBS	PFC6_B	MEANPFC	SE_PC	PFCSPLA	PFCSPLB	MEANSPLN	SE_SPLN	CELLS	SPLEEN
1	682.249	797.7071919	47.9	136.5	108	109.825	6.7	772.27	122.250
2	692.641	.	.	85	80	.	.	714.29	82.500
3	631.51	.	.	109	97	.	.	670.57	103.000
4	709.382	.	.	99	93	.	.	732.27	96.000
5	495.92	.	.	84.5	79	.	.	513.18	81.750
6	1354.44	.	.	170.5	184	.	.	1304.75	177.250
7	827.389	.	.	89.5	116	.	.	732.88	102.750
8	849.019	.	.	102	106	.	.	833.00	104.000
9	890.756	.	.	75	106	.	.	760.50	90.500
10	996.247	.	.	130.5	146	.	.	943.36	138.250
11	1067.52	928.9444814	76.8	142.5	151	130.425	10.8	1037.47	146.750
12	934.635	.	.	79	158	.	.	700.98	118.500
13	1029.66	.	.	97	151	.	.	845.55	124.000
14	1177.23	.	.	251.5	183	.	.	1397.56	217.250
15	1545.1	.	.	182	197	.	.	1486.27	189.500

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40	CCOUNT7	CCOUNT6	VIABIL	PFC6_A
16	7-1	AP-0.2 mg/kg/day	111	115	58	45	2.878	28.78	100	785.268
17	7-2	AP-0.2 mg/kg/day	109	97	26	30	3.016	30.16	99	683.024
18	7-3	AP-0.2 mg/kg/day	84	.	83	64	2.29	22.9	99	733.624
19	7-4	AP-0.2 mg/kg/day	119	87	58	72	2.724	27.24	100	756.241
20	7-5	AP-0.2 mg/kg/day	100	85	41	44	2.611	26.11	100	708.541
21	4-1	AP-2.0 mg/kg/day	104	103	63	50	2.659	26.59	100	778.488
22	4-2	AP-2.0 mg/kg/day	172	206	86	117	3.469	34.69	99	1089.65
23	4-3	AP-2.0 mg/kg/day	142	144	80	86	2.82	28.2	98	1014.18
24	4-4	AP-2.0 mg/kg/day	102	128	57	63	2.969	29.69	98	774.672
25	4-5	AP-2.0 mg/kg/day	99	85	.	54	2.659	26.59	99	691.989
26	5-1	AP-2.0 mg/kg/day	180	192	91	88	2.652	26.52	100	1402.71
27	5-2	AP-2.0 mg/kg/day	203	182	114	99	4.528	45.28	100	850.265
28	5-3	AP-2.0 mg/kg/day	81	104	42	68	2.432	24.32	98	760.691
29	5-4	AP-2.0 mg/kg/day	288	270	129	171	4.11	41.1	100	1357.66
30	5-5	AP-2.0 mg/kg/day	195	178	110	95	3.44	34.4	100	1084.3

OBS	PFC6_B	MEANPFC	SE_PC	PFCSPLA	PFCSPLB	MEANSPLN	SE_SPLN	CELLS	SPLEEN
16	715.775	.	.	113	103	.	.	750.52	108.000
17	371.353	.	.	103	56	.	.	527.19	79.500
18	1283.84	.	.	84	147	.	.	1008.73	115.500
19	954.479	.	.	103	130	.	.	855.36	116.500
20	651.092	.	.	92.5	85	.	.	679.82	88.750
21	849.944	1023.499113	54	103.5	113	164.8	13.3	814.22	108.250
22	1170.37	.	.	189	203	.	.	1130.01	196.000
23	1177.3	.	.	143	166	.	.	1095.74	154.500
24	808.353	.	.	115	120	.	.	791.51	117.500
25	812.335	.	.	92	108	.	.	752.16	100.000
26	1349.92	.	.	186	179	.	.	1376.32	182.500
27	940.813	.	.	192.5	213	.	.	895.54	202.750
28	904.605	.	.	92.5	110	.	.	832.65	101.250
29	1459.85	.	.	279	300	.	.	1408.76	289.500
30	1191.86	.	.	186.5	205	.	.	1138.08	195.750

Print of the dataset

OBS	ANIMAL	TREATMENT	DILP1_20	DILP2_20	DILP1_40	DILP2_40	CCOUNT7	CCOUNT6	VIABIL	PFC6_A
31	2-1	AP-50 mg/kg/day	183	181	97	113	3.493	34.93	98	1042.08
32	2-2	AP-50 mg/kg/day	219	218	140	103	3.019	30.19	96	1447.5
33	2-3	AP-50 mg/kg/day	88	62	42	32	2.678	26.78	99	560.119
34	2-4	AP-50 mg/kg/day	171	129	90	83	3.478	34.78	96	862.565
35	2-5	AP-50 mg/kg/day	123	123	92	77	3.156	31.56	98	779.468
36	3-1	AP-50 mg/kg/day	99	118	45	49	1.996	19.96	98	1087.17
37	3-2	AP-50 mg/kg/day	178	148	93	78	2.799	27.99	98	1164.7
38	3-3	AP-50 mg/kg/day	189	165	79	71	2.514	25.14	100	1408.11
39	3-4	AP-50 mg/kg/day	110	106	40	59	2.739	27.39	98	788.609
40	3-5	AP-50 mg/kg/day	348	362	195	171	3.304	33.04	100	2148.91
41	13-1	Vehicle + CP	92	90	80	55	2.551	25.51	98	356.723
42	13-2	Vehicle + CP	87	85	82	52	3.06	30.6	99	281.046
43	13-3	Vehicle + CP	74	72	52	54	2.668	26.68	96	273.613
44	13-4	Vehicle + CP	280	298	141	162	3.839	38.39	98	752.8
45	13-5	Vehicle + CP	76	84	61	46	3.41	34.1	99	234.604

OBS	PFC6_B	MEANPFC	SE_PC	PFCSPLA	PFCSPLB	MEANSPLN	SE_SPLN	CELLS	SPLEEN
31	1202.4	1150.76802	100	182	210	170.45	17.9	1122.24	196.000
32	1609.8	.	.	218.5	243	.	.	1528.65	230.750
33	552.651	.	.	75	74	.	.	556.39	74.500
34	994.825	.	.	150	173	.	.	928.69	161.500
35	1070.98	.	.	123	169	.	.	925.22	146.000
36	941.884	.	.	108.5	94	.	.	1014.53	101.250
37	1221.86	.	.	163	171	.	.	1193.28	167.000
38	1193.32	.	.	177	150	.	.	1300.72	163.500
39	722.892	.	.	108	99	.	.	755.75	103.500
40	2215.5	.	.	355	366	.	.	2182.20	360.500
41	529.204	413.7114018	38.3	45.5	67.5	60.825	8.35	442.96	56.500
42	437.908	.	.	43	67	.	.	359.48	55.000
43	397.301	.	.	36.5	53	.	.	335.46	44.750
44	789.268	.	.	144.5	151.5	.	.	771.03	148.000
45	313.783	.	.	40	53.5	.	.	274.19	46.750

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1	DILP2	DILP1	DILP2	CCOUNT7	CCOUNT6	VIABIL	PFC6_A
46	14-1	Vehicle + CP	175	176	127	110	3.338	33.38	98	525.764
47	14-2	Vehicle + CP	32	38	35	24	1.696	16.96	99	206.368
48	14-3	Vehicle + CP	49	59	40	48	2.477	24.77	100	218.006
49	14-4	Vehicle + CP	95	101	64	63	2.799	27.99	98	350.125
50	14-5	Vehicle + CP	65	70	41	47	2.099	20.99	100	321.582
51	11-1	Vehicle Control	136	114	87	81	3.25	32.5	100	769.231
52	11-2	Vehicle Control	134	132	77	77	3.049	30.49	97	872.417
53	11-3	Vehicle Control	103	108	42	42	2.847	28.47	98	741.131
54	11-4	Vehicle Control	200	161	103	112	2.895	28.95	98	1246.98
55	11-5	Vehicle Control	146	197	70	71	2.662	26.62	100	1288.5
56	12-1	Vehicle Control	151	158	73	62	2.671	26.71	99	1156.87
57	12-2	Vehicle Control	169	147	72	83	2.856	28.56	99	1106.44
58	12-3	Vehicle Control	145	139	68	79	2.904	29.04	99	977.961
59	12-4	Vehicle Control	197	161	95	86	3.449	34.49	99	1037.98
60	12-5	Vehicle Control	169	139	64	59	3.406	34.06	97	904.287

OBS	PFC6_B	MEANPFC	SE_PC	PFCSPLA	PFCSPLB	MEANSPLN	SE_SPLN	CELLS	SPLEEN
46	710.006	.	.	87.75	118.5	.	.	617.88	103.125
47	347.877	.	.	17.5	29.5	.	.	277.12	23.500
48	355.268	.	.	27	44	.	.	286.64	35.500
49	453.733	.	.	49	63.5	.	.	401.93	56.250
50	419.247	.	.	33.75	44	.	.	370.41	38.875
51	1033.85	1008.055419	46.5	125	168	150.3	6.54	901.54	146.500
52	1010.17	.	.	133	154	.	.	941.29	143.500
53	590.095	.	.	105.5	84	.	.	665.61	94.750
54	1485.32	.	.	180.5	215	.	.	1366.15	197.750
55	1059.35	.	.	171.5	141	.	.	1173.93	156.250
56	1010.86	.	.	154.5	135	.	.	1083.86	144.750
57	1085.43	.	.	158	155	.	.	1095.94	156.500
58	1012.4	.	.	142	147	.	.	995.18	144.500
59	1049.58	.	.	179	181	.	.	1043.78	180.000
60	722.255	.	.	154	123	.	.	813.27	138.500

4) EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice

1

Test for Pooling Low and High Dose Studies

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	2	AP-0 AP-0 + CP
DATAST	2	Hi Low

Number of observations in data set = 40

Test for Pooling Low and High Dose Studies

General Linear Models Procedure

Dependent Variable: CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	7168206.28715878	2389402.09571959	14.13	0.0001
Error	36	6089393.09864432	169149.80829568		
Corrected Total	39	13257599.38580300			

R-Square	C.V.	Root MSE	CELLS Mean
0.540687	47.63089	411.27826139	863.46959231

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	5286126.16595172	5286126.16595172	31.25	0.0001
DATAST	1	386836.78403421	386836.78403421	2.29	0.1392
TREATMEN*DATAST	1	1495243.33717284	1495243.33717284	8.84	0.0052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	3477428.86841942	3477428.86841942	20.56	0.0001
DATAST	1	893794.95982141	893794.95982141	5.28	0.0274
TREATMEN*DATAST	1	1495243.33717284	1495243.33717284	8.84	0.0052

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
dataset	1	53863.25297594	53863.25297594	0.32	0.5760

Test for Pooling Low and High Dose Studies

General Linear Models Procedure

Dependent Variable: SPLEEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	255909.20625000	85303.06875000	12.62	0.0001
Error	36	243401.57500000	6761.15486111		
Corrected Total	39	499310.78125000			

R-Square	C.V.	Root MSE	SPLEEN Mean
0.512525	55.44121	82.22624193	148.31250000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	201428.40375000	201428.40375000	29.79	0.0001
DATAST	1	0.15428571	0.15428571	0.00	0.9962
TREATMEN*DATAST	1	54480.64821429	54480.64821429	8.06	0.0074

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	156735.43392857	156735.43392857	23.18	0.0001
DATAST	1	4397.33571429	4397.33571429	0.65	0.4253
TREATMEN*DATAST	1	54480.64821429	54480.64821429	8.06	0.0074

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
dataset	1	19545.33375000	19545.33375000	2.89	0.0977

Test for Pooling Low and High Dose Studies

SUMMARY STATISTICS FOR DATAST BY CELLS
CONTROLLING FOR TREATMEN

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.835	0.176
2	Row Mean Scores Differ	1	1.835	0.176
3	General Association	39	.	.

At least 1 statistic not computed--singular covariance matrix.

Total Sample Size = 40

Test for Pooling Low and High Dose Studies

SUMMARY STATISTICS FOR DATAST BY SPLEEN
CONTROLLING FOR TREATMEN

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.000	0.996
2	Row Mean Scores Differ	1	0.000	0.996
3	General Association	37	.	.

At least 1 statistic not computed--singular covariance matrix.

Total Sample Size = 40

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	2	AP-0 AP-0 + CP

Number of observations in data set = 40

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure

Dependent Variable: CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5286126.16595172	5286126.16595172	25.20	0.0001
Error	38	7971473.21985137	209775.61104872		
Corrected Total	39	13257599.38580300			

R-Square	C.V.	Root MSE	CELLS Mean
0.398724	53.04329	458.01267564	863.46959231

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	5286126.16595172	5286126.16595172	25.20	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	5286126.16595172	5286126.16595172	25.20	0.0001

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure

Dependent Variable: SPLEEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	201428.40375000	201428.40375000	25.70	0.0001
Error	38	297882.37750000	7839.00993421		
Corrected Total	39	499310.78125000			

R-Square	C.V.	Root MSE	SPLEEN Mean
0.403413	59.69705	88.53818348	148.31250000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	1	201428.40375000	201428.40375000	25.70	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	1	201428.40375000	201428.40375000	25.70	0.0001

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure

Bartlett's Test for Equality
of CELLS Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	1	0.8987	0.3431

Bartlett's Test for Equality
of SPLEEN Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	1	5.2059	0.0225

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure

Dunnett's T tests for variable: CELLS

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 38 MSE= 209775.6
Critical Value of Dunnett's T= 2.024

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
AP-0 + CP - AP-0	-1053.7	-750.9	-448.1	***

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Control

General Linear Models Procedure

Dunnett's T tests for variable: SPLEEN

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 38 MSE= 7839.01
Critical Value of Dunnett's T= 2.024

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
AP-0 + CP - AP-0	-205.12	-146.58	-88.04	***

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	6	AP-0 AP-0.02 AP-0.06 AP-0.2 AP-2 AP-50 mg/kg/day

Number of observations in data set = 75

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure

Dependent Variable: CELLS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6351247.38421939	1270249.47684388	6.40	0.0001
Error	69	13691319.27108550	198424.91697225		
Corrected Total	74	20042566.65530490			

R-Square	C.V.	Root MSE	CELLS Mean
0.316888	34.23844	445.44911828	1301.02062761

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	5	6351247.38421940	1270249.47684388	6.40	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	5	6351247.38421939	1270249.47684388	6.40	0.0001

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure

Dependent Variable: SPLEEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	221130.85500000	44226.17100000	4.90	0.0007
Error	69	622337.48375000	9019.38382246		
Corrected Total	74	843468.33875000			

R-Square	C.V.	Root MSE	SPLEEN Mean
0.262169	39.70004	94.97043657	239.22000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	5	221130.85500000	44226.17100000	4.90	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	5	221130.85500000	44226.17100000	4.90	0.0007

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure

Bartlett's Test for Equality
of CELLS Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	5	6.4382	0.2659

Bartlett's Test for Equality
of SPLEEN Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	5	5.4247	0.3663

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure

Dunnett's T tests for variable: CELLS

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 69 MSE= 198424.9
Critical Value of Dunnett's T= 2.618

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
AP-50 mg/kg/day	- AP-0	378.9	815.3	1251.7	***
AP-2	- AP-0	-49.8	386.6	823.1	
AP-0.06	- AP-0	-419.2	17.2	453.7	
AP-0.2	- AP-0	-431.1	5.3	441.7	
AP-0.02	- AP-0	-491.2	-54.8	381.6	

One-Way ANOVA on EPA / Air Force 90 Day Plaque-Forming Cell (PFC) Assay in Mice
Test of Immunosuppression

General Linear Models Procedure

Dunnett's T tests for variable: SPLEEN

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 69 MSE= 9019.384
Critical Value of Dunnett's T= 2.618

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
AP-50 mg/kg/day	- AP-0	49.27	142.32	235.37	***
AP-2	- AP-0	8.97	102.02	195.07	***
AP-0.2	- AP-0	-63.93	29.12	122.17	
AP-0.06	- AP-0	-83.13	9.92	102.97	
AP-0.02	- AP-0	-106.88	-13.83	79.22	

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
1	2-1	AP-0	262	295	164	148
2	2-2	AP-0	446	400	229	197
3	2-3	AP-0	323	289	121	119
4	2-4	AP-0	322	260	120	112
5	2-5	AP-0	105	141	57	59
6	3-1	AP-0	222	239	105	115
7	3-2	AP-0	131	128	59	73
8	3-3	AP-0	268	222	151	136
9	3-4	AP-0	353	339	178	130
10	3-5	AP-0	57	51	27	24
11	7-1	AP-0	.	.	178	149
12	7-2	AP-0	211	231	100	98
13	7-3	AP-0	252	211	110	103
14	7-4	AP-0	121	137	61	81

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
1	4.103	41.03	94	1357.54	1520.84	1201.906634	92.9	278.5
2	4.554	45.54	94	1857.71	1870.88	.	.	423
3	4.472	44.72	93	1368.52	1073.35	.	.	306
4	4.28	42.8	95	1359.81	1084.11	.	.	291
5	3.134	31.34	95	784.939	740.268	.	.	123
6	3.466	34.66	98	1330.06	1269.47	.	.	230.5
7	2.267	22.67	94	1142.48	1164.53	.	.	129.5
8	4.736	47.36	97	1034.63	1211.99	.	.	245
9	4.135	41.35	97	1673.52	1489.72	.	.	346
10	2.984	29.84	94	361.93	341.823	.	.	54
11	3.374	33.74	97	.	1938.35	1243.681274	124	.
12	2.885	28.85	98	1532.06	1372.62	.	.	221
13	2.889	28.89	97	1602.63	1474.56	.	.	231.5
14	2.389	23.89	97	1079.95	1188.78	.	.	129

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
1	312	237.525	24.3	Low	1439.19	295.250
2	426	.	.	Low	1864.30	424.500
3	240	.	.	Low	1220.93	273.000
4	232	.	.	Low	1221.96	261.500
5	116	.	.	Low	762.60	119.500
6	220	.	.	Low	1299.77	225.250
7	132	.	.	Low	1153.51	130.750
8	287	.	.	Low	1123.31	266.000
9	308	.	.	Low	1581.62	327.000
10	51	.	.	Low	351.88	52.500
11	327	196.4705882	19.6	Hi	1938.35	327.000
12	198	.	.	Hi	1452.34	209.500
13	213	.	.	Hi	1538.59	222.250
14	142	.	.	Hi	1134.37	135.500

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
15	7-5	AP-0	254	190	115	102
16	8-1	AP-0	.	.	145	190
17	8-2	AP-0	.	.	162	165
18	8-3	AP-0	159	218	70	84
19	8-4	AP-0	53	63	34	43
20	8-5	AP-0	137	111	81	95
21	5-1	AP-0	.	.	123	137
22	5-2	AP-0	240	190	121	94
23	5-3	AP-0	81	66	46	43
24	5-4	AP-0	134	149	93	80
25	5-5	AP-0	.	.	188	183
26	17-1	AP-0 + CP	87	89	29	30
27	17-2	AP-0 + CP	60	52	18	30
28	17-3	AP-0 + CP	55	38	27	21

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
15	4.67	46.7	99	950.749	929.336	.	.	222
16	3.391	33.91	97	.	1975.82	.	.	.
17	4.496	44.96	96	.	1454.63	.	.	.
18	2.068	20.68	98	1823.02	1489.36	.	.	188.5
19	5.337	53.37	98	217.351	288.552	.	.	58
20	3.288	32.88	98	754.258	1070.56	.	.	124
21	3.222	32.22	97	.	806.952	.	.	.
22	3.695	36.95	98	581.867	581.867	.	.	107.5
23	2.406	24.06	98	305.486	369.909	.	.	36.75
24	2.726	27.26	96	519.076	634.629	.	.	70.75
25	3.539	35.39	98	.	1048.32	.	.	.
26	3.061	30.61	96	287.488	192.747	147.2945672	17	44
27	2.855	28.55	98	196.147	168.126	.	.	28
28	2.374	23.74	97	195.872	202.19	.	.	23.25

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
15	217	.	.	Hi	940.04	219.500
16	335	.	.	Hi	1975.82	335.000
17	327	.	.	Hi	1454.63	327.000
18	154	.	.	Hi	1656.19	171.250
19	77	.	.	Hi	252.95	67.500
20	176	.	.	Hi	912.41	150.000
21	130	.	.	Hi	806.95	130.000
22	107.5	.	.	Hi	581.87	107.500
23	44.5	.	.	Hi	337.70	40.625
24	86.5	.	.	Hi	576.85	78.625
25	185.5	.	.	Hi	1048.32	185.500
26	29.5	22.575	2.92	Low	240.12	36.750
27	24	.	.	Low	182.14	26.000
28	24	.	.	Low	199.03	23.625

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
29	17-4	AP-0 + CP	20	25	12	18
30	17-5	AP-0 + CP	67	62	42	32
31	18-1	AP-0 + CP	24	20	2	6
32	18-2	AP-0 + CP	90	72	46	33
33	18-3	AP-0 + CP	28	12	5	13
34	18-4	AP-0 + CP	83	71	14	20
35	18-5	AP-0 + CP	19	12	5	7
36	4-1	AP-0 + CP	.	.	196	187
37	4-2	AP-0 + CP	149	138	66	81
38	4-3	AP-0 + CP	280	293	175	147
39	4-4	AP-0 + CP	207	181	112	114
40	4-5	AP-0 + CP	195	191	95	126
41	5-1	AP-0.02	.	.	153	152
42	5-2	AP-0.02	.	.	116	97

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
29	2.995	29.95	95	75.1252	100.167	.	.	11.25
30	3.649	36.49	98	176.761	202.795	.	.	32.25
31	2.43	24.3	100	90.535	32.9218	.	.	11
32	3.733	37.33	97	216.984	211.626	.	.	40.5
33	2.992	29.92	98	66.8449	60.1604	.	.	10
34	3.1	31	98	248.387	109.677	.	.	38.5
35	2.47	24.7	97	62.753	48.583	.	.	7.75
36	3.083	30.83	98	.	1242.3	734.3880898	61.7	.
37	2.562	25.62	99	560.109	573.77	.	.	71.75
38	3.001	30.01	99	954.682	1072.98	.	.	143.25
39	2.55	25.5	97	760.784	886.275	.	.	97
40	2.611	26.11	98	739.18	846.419	.	.	96.5
41	3.685	36.85	93	.	1655.36	1000.26703	107	.
42	3.478	34.78	96	.	1224.84	.	.	.

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
29	15	.	.	Low	87.65	13.125
30	37	.	.	Low	189.78	34.625
31	4	.	.	Low	61.73	7.500
32	39.5	.	.	Low	214.30	40.000
33	9	.	.	Low	63.50	9.500
34	17	.	.	Low	179.03	27.750
35	6	.	.	Low	55.67	6.875
36	191.5	107.4705882	10.7	Hi	1242.30	191.500
37	73.5	.	.	Hi	566.94	72.625
38	161	.	.	Hi	1013.83	152.125
39	113	.	.	Hi	823.53	105.000
40	110.5	.	.	Hi	792.80	103.500
41	305	166.9333333	21.5	Low	1655.36	305.000
42	213	.	.	Low	1224.84	213.000

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
43	5-3	AP-0.02	156	160	60	60
44	5-4	AP-0.02	.	.	112	99
45	5-5	AP-0.02	106	74	39	52
46	6-1	AP-0.02	.	.	112	110
47	6-2	AP-0.02	200	212	93	85
48	6-3	AP-0.02	.	.	176	158
49	6-4	AP-0.02	140	147	45	45
50	6-5	AP-0.02	97	114	22	15
51	8-1	AP-0.06	.	.	158	190
52	8-2	AP-0.06	.	.	96	106
53	8-3	AP-0.06	.	.	122	122
54	8-4	AP-0.06	193	178	67	80
55	8-5	AP-0.06	181	211	70	73
56	9-1	AP-0.06	314	.	147	92

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
43	2.868	28.68	96	1101.81	836.82	.	.	158
44	4.683	46.83	93	.	901.132	.	.	.
45	2.801	28.01	97	642.628	649.768	.	.	90
46	3.014	30.14	95	.	1473.13	.	.	.
47	2.986	29.86	96	1379.77	1192.23	.	.	206
48	4.318	43.18	95	.	1547.01	.	.	.
49	2.917	29.17	97	983.888	617.072	.	.	143.5
50	3.569	35.69	97	591.202	207.341	.	.	105.5
51	4.339	43.39	94	.	1604.06	1149.138117	73.4	.
52	4.685	46.85	93	.	862.327	.	.	.
53	3.466	34.66	93	.	1407.96	.	.	.
54	3.621	36.21	98	1024.58	811.93	.	.	185.5
55	3.519	35.19	95	1113.95	812.731	.	.	196
56	3.752	37.52	98	1673.77	1273.99	.	.	314

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
43	120	.	.	Low	969.32	139.00
44	211	.	.	Low	901.13	211.00
45	91	.	.	Low	646.20	90.50
46	222	.	.	Low	1473.13	222.00
47	178	.	.	Low	1286.00	192.00
48	334	.	.	Low	1547.01	334.00
49	90	.	.	Low	800.48	116.75
50	37	.	.	Low	399.27	71.25
51	348	208.0714286	16.2	Low	1604.06	348.00
52	202	.	.	Low	862.33	202.00
53	244	.	.	Low	1407.96	244.00
54	147	.	.	Low	918.25	166.25
55	143	.	.	Low	963.34	169.50
56	239	.	.	Low	1473.88	276.50

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
57	9-2	AP-0.06	200	189	85	58
58	9-3	AP-0.06	.	.	94	93
59	9-4	AP-0.06	.	.	89	107
60	9-5	AP-0.06	.	.	101	73
61	11-1	AP-0.2	267	342	126	95
62	11-2	AP-0.2	.	.	177	154
63	11-3	AP-0.2	.	.	183	136
64	11-4	AP-0.2	.	.	95	106
65	11-5	AP-0.2	.	.	147	175
66	12-1	AP-0.2	155	107	49	35
67	12-2	AP-0.2	211	269	47	71
68	12-3	AP-0.2	.	.	89	92
69	12-4	AP-0.2	.	.	118	114
70	12-5	AP-0.2	214	249	80	66

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
57	3.042	30.42	97	1278.76	940.171	.	.	194.5
58	3.176	31.76	99	.	1177.58	.	.	.
59	3.487	34.87	96	.	1124.18	.	.	.
60	3.544	35.44	92	.	981.941	.	.	.
61	3.602	36.02	97	1690.73	1227.1	1095.511819	105	304.5
62	4.67	46.7	98	.	1417.56	.	.	.
63	3.671	36.71	96	.	1737.95	.	.	.
64	3.763	37.63	96	.	1068.3	.	.	.
65	4.274	42.74	97	.	1506.79	.	.	.
66	3.342	33.42	97	783.962	502.693	.	.	131
67	4.114	41.14	97	1166.75	573.651	.	.	240
68	4.172	41.72	99	.	867.689	.	.	.
69	4.329	43.29	96	.	1071.84	.	.	.
70	4.384	43.84	96	1056.11	666.058	.	.	231.5

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
57	143	.	.	Low	1109.47	168.75
58	187	.	.	Low	1177.58	187.00
59	196	.	.	Low	1124.18	196.00
60	174	.	.	Low	981.94	174.00
61	221	218.7142857	21.5	Low	1458.91	262.75
62	331	.	.	Low	1417.56	331.00
63	319	.	.	Low	1737.95	319.00
64	201	.	.	Low	1068.30	201.00
65	322	.	.	Low	1506.79	322.00
66	84	.	.	Low	643.33	107.50
67	118	.	.	Low	870.20	179.00
68	181	.	.	Low	867.69	181.00
69	232	.	.	Low	1071.84	232.00
70	146	.	.	Low	861.09	188.75

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
71	14-1	AP-2	.	.	104	88
72	14-2	AP-2	.	.	185	157
73	14-3	AP-2	.	.	197	148
74	14-4	AP-2	.	.	97	59
75	14-5	AP-2	.	.	163	176
76	15-1	AP-2	.	.	246	259
77	15-2	AP-2	.	.	149	136
78	15-3	AP-2	.	.	171	162
79	15-4	AP-2	.	.	135	188
80	15-5	AP-2	.	.	129	104
81	2-1	AP-50 mg/kg/day	.	.	238	291
82	2-2	AP-50 mg/kg/day	.	.	180	155
83	2-3	AP-50 mg/kg/day	.	.	159	159
84	2-4	AP-50 mg/kg/day	.	.	241	220

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
71	3.314	33.14	98	.	1158.72	1531.700862	137	.
72	4.214	42.14	96	.	1623.16	.	.	.
73	3.934	39.34	98	.	1753.94	.	.	.
74	3.605	36.05	97	.	865.465	.	.	.
75	3.63	36.3	99	.	1867.77	.	.	.
76	4.18	41.8	98	.	2416.27	.	.	.
77	3.615	36.15	97	.	1576.76	.	.	.
78	4.403	44.03	99	.	1512.61	.	.	.
79	4.74	47.4	97	.	1362.87	.	.	.
80	3.951	39.51	93	.	1179.45	.	.	.
81	5.171	51.71	97	.	2046.03	1896.92091	155	.
82	3.529	35.29	99	.	1898.55	.	.	.
83	3.424	34.24	97	.	1857.48	.	.	.
84	4.132	41.32	99	.	2231.36	.	.	.

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
71	192	305.3	30.9	Low	1158.72	192.00
72	342	.	.	Low	1623.16	342.00
73	345	.	.	Low	1753.94	345.00
74	156	.	.	Low	865.46	156.00
75	339	.	.	Low	1867.77	339.00
76	505	.	.	Low	2416.27	505.00
77	285	.	.	Low	1576.76	285.00
78	333	.	.	Low	1512.61	333.00
79	323	.	.	Low	1362.87	323.00
80	233	.	.	Low	1179.45	233.00
81	529	325.8461538	32	Hi	2046.03	529.00
82	335	.	.	Hi	1898.55	335.00
83	318	.	.	Hi	1857.48	318.00
84	461	.	.	Hi	2231.36	461.00

Print of the dataset

OBS	ANIMAL	TREATMEN	DILP1_20	DILP2_20	DILP1_40	DILP2_40
85	2-5	AP-50 mg/kg/day	.	.	126	115
86	3-1	AP-50 mg/kg/day	.	.	127	128
87	3-2	AP-50 mg/kg/day	.	340	168	158
88	3-3	AP-50 mg/kg/day	249	199	105	141
89	3-4	AP-50 mg/kg/day	.	.	249	288
90	3-5	AP-50 mg/kg/day	190	196	115	116

OBS	CCOUNT7	CCOUNT6	VIABIL	PFC6_A	PFC6_B	MEANPFC	SE_PC	PFCSPLA
85	2.983	29.83	97	.	1615.82	.	.	.
86	3.133	31.33	97	.	1627.83	.	.	.
87	2.859	28.59	94	2378.45	2280.52	.	.	340
88	3.514	35.14	97	1274.9	1400.11	.	.	224
89	3.284	32.84	98	.	3270.4	.	.	.
90	3.052	30.52	97	1264.74	1513.76	.	.	193

OBS	PFCSPLB	MEANSPLN	SE_SPLN	DATAST	CELLS	SPLEEN
85	241	.	.	Hi	1615.82	241.00
86	255	.	.	Hi	1627.83	255.00
87	326	.	.	Hi	2329.49	333.00
88	246	.	.	Hi	1337.51	235.00
89	537	.	.	Hi	3270.40	537.00
90	231	.	.	Hi	1389.25	212.00

Test for each compound dose SI greater than 3

Analysis Variable : SI_3

Treatment=AP 0.06 mg/kg

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	51.1831395	4.3275590	19.6046512	65.9127907	11.8272541	0.0001

Treatment=AP 0.2 mg/kg

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
9	55.7383721	5.9624389	25.6220930	85.0116279	9.3482504	0.0001

Treatment=AP 2.0 mg/kg

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
9	47.2209302	7.9766854	16.2034884	94.1686047	5.9198687	0.0004

Treatment=AP 50 mg/kg

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
8	57.2136628	6.2399666	21.6976744	76.9011628	9.1689053	0.0001

Treatment=DNCB Control

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	29.9127907	4.7073963	10.1860465	59.1104651	6.3544238	0.0001

Treatment=DNCB Control - CP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	9.1395349	2.5702344	-1.3255814	25.0988372	3.5559150	0.0062

Test for each compound dose SI greater than 3

Analysis Variable : SI_3

Treatment=Vehicle Control

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
0	.	.	.	.	.	.

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
DNCB Control	10	159.50297959	72.14144712	22.81312866	6.39026E+01	301.00164828
Vehicle Control	9	4.84623079	6.60463203	2.20154401	0.00000E+00	18.25789273

Variances	T	DF	Prob>|T|
Unequal	6.7479	9.2	0.0001
Equal	6.3888	17.0	0.0000

For H0: Variances are equal, F' = 119.31 DF = (9,8) Prob>F' = 0.0000

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
DNCB Control	10	159.50297959	72.14144712	22.81312866	63.90262457	301.00164828
DNCB Control - C	10	58.83098770	39.38916886	12.45594887	8.11461899	136.17344998

Variances	T	DF	Prob>|T|
Unequal	3.8732	13.9	0.0017
Equal	3.8732	18.0	0.0011

For H0: Variances are equal, F' = 3.35 DF = (9,9) Prob>F' = 0.0859

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
AP 0.06 mg/kg	10	262.58399899	66.32039246	20.97234955	109.54735641	333.96728794
DNCB Control	10	159.50297959	72.14144712	22.81312866	63.90262457	301.00164828

Variances	T	DF	Prob>|T|
Unequal	3.3264	17.9	0.0038
Equal	3.3264	18.0	0.0038

For HO: Variances are equal, F' = 1.18 DF = (9,9) Prob>F' = 0.8062

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
AP 0.2 mg/kg	9	284.65970725	86.68606502	28.89535501	138.70926842	426.52466083
DNCB Control	10	159.50297959	72.14144712	22.81312866	63.90262457	301.00164828

Variances	T	DF	Prob>|T|
Unequal	3.3996	15.7	0.0038
Equal	3.4342	17.0	0.0032

For HO: Variances are equal, F' = 1.44 DF = (8,9) Prob>F' = 0.5939

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
AP 2.0 mg/kg	9	243.38221828	115.97057495	38.65685832	93.06453658	470.90148345
DNCB Control	10	159.50297959	72.14144712	22.81312866	63.90262457	301.00164828

Variances	T	DF	Prob>|T|
Unequal	1.8687	13.1	0.0842
Equal	1.9154	17.0	0.0724

For H0: Variances are equal, F' = 2.58 DF = (8,9) Prob>F' = 0.1790

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
AP 50 mg/kg	8	291.80930645	85.53253666	30.24031834	119.69063015	387.21947509
DNCB Control	10	159.50297959	72.14144712	22.81312866	63.90262457	301.00164828

Variances	T	DF	Prob>|T|
Unequal	3.4927	13.8	0.0037
Equal	3.5631	16.0	0.0026

For H0: Variances are equal, F' = 1.41 DF = (7,9) Prob>F' = 0.6203

One-Way ANOVA on EPA / Air Force 14 Day LLNA

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	5	AP 0.06 mg/kg AP 0.2 mg/kg AP 2.0 mg/kg AP 50 mg/kg DNCB Control

Number of observations in data set = 50

NOTE: Due to missing values, only 46 observations can be used in this analysis.

One-Way ANOVA on EPA / Air Force 14 Day LLNA

General Linear Models Procedure

Dependent Variable: DPM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	107866.91796855	26966.72949214	3.62	0.0129
Error	41	305344.93442313	7447.43742495		
Corrected Total	45	413211.85239169			

R-Square	C.V.	Root MSE	DPM Mean
0.261045	35.10639	86.29853663	245.82003407

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	4	107866.91796855	26966.72949214	3.62	0.0129

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	4	107866.91796855	26966.72949214	3.62	0.0129

One-Way ANOVA on EPA / Air Force 14 Day LLNA

General Linear Models Procedure

Bartlett's Test for Equality of DPM Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	4	3.1354	0.5354

One-Way ANOVA on EPA / Air Force 14 Day LLNA

General Linear Models Procedure

Dunnett's T tests for variable: DPM

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 41 MSE= 7447.437
Critical Value of Dunnett's T= 2.547

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
AP 50 mg/kg - DNCB Control	28.03	132.31	236.58	***
AP 0.2 mg/kg - DNCB Control	24.15	125.16	226.16	***
AP 0.06 mg/kg - DNCB Control	4.77	103.08	201.39	***
AP 2.0 mg/kg - DNCB Control	-17.12	83.88	184.88	

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM
1	AP 0.06 mg/kg	188	235	262.583999
2	AP 0.06 mg/kg	266	334	
3	AP 0.06 mg/kg	230	288	
4	AP 0.06 mg/kg	249	312	
5	AP 0.06 mg/kg	247	310	
6	AP 0.06 mg/kg	214	268	
7	AP 0.06 mg/kg	219	274	
8	AP 0.06 mg/kg	234	293	
9	AP 0.06 mg/kg	161	201	
10	AP 0.06 mg/kg	89	110	
11	AP 0.2 mg/kg	339	427	284.6597073
12	AP 0.2 mg/kg	191	239	
13	AP 0.2 mg/kg	211	264	
14	AP 0.2 mg/kg	214	268	
15	AP 0.2 mg/kg	112	139	
16	AP 0.2 mg/kg	185	231	
17	AP 0.2 mg/kg	223	279	
18	AP 0.2 mg/kg	.	.	
19	AP 0.2 mg/kg	312	392	
20	AP 0.2 mg/kg	257	323	
21	AP 2.0 mg/kg	173	216	243.3822183
22	AP 2.0 mg/kg	250	314	

OBS	SE	SI	GROUP_SI	SE_1	DOSE
1	20.97	48.505813953	54.18314	4.328	0.06
2	.	68.912790698	.	.	0.06
3	.	59.494186047	.	.	0.06
4	.	64.465116279	.	.	0.06
5	.	63.941860465	.	.	0.06
6	.	55.308139535	.	.	0.06
7	.	56.61627907	.	.	0.06
8	.	60.540697674	.	.	0.06
9	.	41.441860465	.	.	0.06
10	.	22.604651163	.	.	0.06
11	28.9	88.011627907	58.738372	.	0.20
12	.	49.290697674	.	.	0.20
13	.	54.523255814	.	.	0.20
14	.	55.308139535	.	.	0.20
15	.	28.622093023	.	.	0.20
16	.	47.720930233	.	.	0.20
17	.	57.662790698	.	.	0.20
18	.	.	.	.	0.20
19	.	80.947674419	.	.	0.20
20	.	66.558139535	.	.	0.20
21	38.66	44.581395349	50.22093	.	2.00
22	.	64.726744186	.	.	2.00

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM
23	AP 2.0 mg/kg	248	311	
24	AP 2.0 mg/kg	217	272	
25	AP 2.0 mg/kg	176	220	
26	AP 2.0 mg/kg	76	93	
27	AP 2.0 mg/kg	88	108	
28	AP 2.0 mg/kg	374	471	
29	AP 2.0 mg/kg	149	186	
30	AP 2.0 mg/kg	.	.	
31	AP 50 mg/kg	97	120	291.8093065
32	AP 50 mg/kg	237	297	
33	AP 50 mg/kg	276	347	
34	AP 50 mg/kg	184	230	
35	AP 50 mg/kg	215	269	
36	AP 50 mg/kg	.	.	
37	AP 50 mg/kg	.	.	
38	AP 50 mg/kg	269	338	
39	AP 50 mg/kg	276	347	
40	AP 50 mg/kg	308	387	
41	DNCB Control	173	216	159.5029796
42	DNCB Control	66	80	
43	DNCB Control	53	64	
44	DNCB Control	137	170	

OBS	SE	SI	GROUP_SI	SE_1	DOSE
23	.	64.203488372	.	.	2
24	.	56.093023256	.	.	2
25	.	45.36627907	.	.	2
26	.	19.203488372	.	.	2
27	.	22.343023256	.	.	2
28	.	97.168604651	.	.	2
29	.	38.302325581	.	.	2
30	.	.	.	.	2
31	30.24	24.697674419	60.213663	.	50
32	.	61.325581395	.	.	50
33	.	71.529069767	.	.	50
34	.	47.459302326	.	.	50
35	.	55.569767442	.	.	50
36	.	.	.	.	50
37	.	.	.	.	50
38	.	69.697674419	.	.	50
39	.	71.529069767	.	.	50
40	.	79.901162791	.	.	50
41	22.81	44.581395349	32.912791	4.707	0
42	.	16.587209302	.	.	0
43	.	13.186046512	.	.	0
44	.	35.162790698	.	.	0

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM
45	DNCB Control	110	136	
46	DNCB Control	240	301	
47	DNCB Control	82	101	
48	DNCB Control	171	214	
49	DNCB Control	107	132	
50	DNCB Control	145	181	
51	DNCB Control - CP	26	30	58.8309877
52	DNCB Control - CP	88	108	
53	DNCB Control - CP	43	51	
54	DNCB Control - CP	41	49	
55	DNCB Control - CP	50	60	
56	DNCB Control - CP	30	35	
57	DNCB Control - CP	26	30	
58	DNCB Control - CP	67	82	
59	DNCB Control - CP	9	8	
60	DNCB Control - CP	110	136	
61	Vehicle Control	12	12	4.8462308
62	Vehicle Control	3	1	
63	Vehicle Control	6	4	
64	Vehicle Control	9	8	
65	Vehicle Control	3	1	
66	Vehicle Control	.	.	

OBS	SE	SI	GROUP_SI	SE_1	DOSE
45	.	28.098837209	.	.	0
46	.	62.110465116	.	.	0
47	.	20.773255814	.	.	0
48	.	44.058139535	.	.	0
49	.	27.313953488	.	.	0
50	.	37.255813953	.	.	0
51	12.46	6.1220930233	12.139535	2.57	.
52	.	22.343023256	.	.	.
53	.	10.569767442	.	.	.
54	.	10.046511628	.	.	.
55	.	12.401162791	.	.	.
56	.	7.1686046512	.	.	.
57	.	6.1220930233	.	.	.
58	.	16.848837209	.	.	.
59	.	1.6744186047	.	.	.
60	.	28.098837209	.	.	.
61	2.202	.	.	.	-1
62	.	.	.	.	-1
63	.	.	.	.	-1
64	.	.	.	.	-1
65	.	.	.	.	-1
66	.	.	.	.	-1

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM
67	Vehicle Control	0	0	
68	Vehicle Control	0	0	
69	Vehicle Control	1	0	
70	Vehicle Control	17	18	

OBS	SE	SI	GROUP_SI	SE_1	DOSE
67	.	.	.	.	-1
68	.	.	.	.	-1
69	.	.	.	.	-1
70	.	.	.	.	-1

Test for Pooling Low and High Dose Studies

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	3	DNCB DNCB + CP Vehicle
DATAST	2	Hi Low

Number of observations in data set = 60

NOTE: Due to missing values, only 58 observations can be used in this analysis.

Test for Pooling Low and High Dose Studies

General Linear Models Procedure

Dependent Variable: DPM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	597916.40774236	119583.28154847	12.25	0.0001
Error	52	507817.29664607	9765.71724319		
Corrected Total	57	1105733.70438844			

R-Square	C.V.	Root MSE	DPM Mean
0.540742	62.50054	98.82164360	158.11326832

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	2	592166.59711510	296083.29855755	30.32	0.0001
DATAST	1	233.69355732	233.69355732	0.02	0.8777
TREATMEN*DATAST	2	5516.11706994	2758.05853497	0.28	0.7551

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	2	594108.20267464	297054.10133732	30.42	0.0001
DATAST	1	291.28809313	291.28809313	0.03	0.8636
TREATMEN*DATAST	2	5516.11706994	2758.05853497	0.28	0.7551

Test for Pooling Low and High Dose Studies

SUMMARY STATISTICS FOR DATAST BY DPM
CONTROLLING FOR DOSE

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	0.025	0.874
2	Row Mean Scores Differ	1	0.025	0.874
3	General Association	48	.	.

At least 1 statistic not computed--singular covariance matrix.

Frequency Missing = 2 Effective Sample Size = 58

Test for each compound dose SI greater than 3

Analysis Variable : SI_3

Treatment=0.02 AP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	9.2131148	1.0144730	3.9063232	15.1943794	9.0816756	0.0001

Treatment=0.06 AP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	11.9812646	0.8764388	8.4964871	16.1311475	13.6703947	0.0001

Treatment=0.2 AP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	11.9391101	2.1464424	1.4238876	24.1873536	5.5622784	0.0004

Treatment=2 AP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
10	9.2084309	0.8910739	4.6557377	13.2271663	10.3340817	0.0001

Treatment=AP-50mg/Kg

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
9	69.2232143	22.2590647	5.9196429	201.9910714	3.1098887	0.0144

Treatment=DNCB

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
20	83.1985509	24.5273214	1.9859485	377.9732143	3.3920765	0.0031

Test for each compound dose SI greater than 3

Analysis Variable : SI_3

Treatment=DNCB + CP

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
19	62.9535699	15.8370458	0.3466042	215.6517857	3.9750829	0.0009

Treatment=Vehicle

N	Mean	Std Error	Minimum	Maximum	T	Prob>|T|
9	-2.0000000	0.9899950	-3.0000000	5.9196429	-2.0202122	0.0780

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
DNCB	20	245.62712343	134.02013272	29.96781271	4.79366E+01	596.50226472
Vehicle	19	14.94035145	23.20979137	5.32469132	0.00000E+00	87.75587566

Variances	T	DF	Prob>|T|
Unequal	7.5791	20.2	0.0001
Equal	7.3935	37.0	0.0000

For H0: Variances are equal, F' = 33.34 DF = (19,18) Prob>F' = 0.0000

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472
DNCB + CP	19	209.16633771	95.04504320	21.80482834	90.28304271	445.35001264

Variances	T	DF	Prob>|T|
Unequal	0.9838	34.3	0.3321
Equal	0.9753	37.0	0.3358

For H0: Variances are equal, F' = 1.99 DF = (19,18) Prob>F' = 0.1512

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
0.02 AP	10	329.47940359	86.54506893	27.36795381	186.31539045	490.83901946
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472

Variances	T	DF	Prob>|T|
Unequal	2.0661	25.9	0.0490
Equal	1.7921	28.0	0.0839

For H0: Variances are equal, F' = 2.40 DF = (19,9) Prob>F' = 0.1799

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
0.06 AP	10	404.15718979	74.76932150	23.64413550	310.14657569	516.11068992
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472

Variances	T	DF	Prob>|T|
Unequal	4.1530	27.5	0.0003
Equal	3.4613	28.0	0.0017

For HO: Variances are equal, F' = 3.21 DF = (19,9) Prob>F' = 0.0770

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
0.2 AP	10	403.01996462	183.11379750	57.90566711	119.34546374	733.44705585
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472

Variances	T	DF	Prob>|T|
Unequal	2.4140	14.0	0.0301
Equal	2.6816	28.0	0.0121

For H0: Variances are equal, F' = 1.87 DF = (9,19) Prob>F' = 0.2413

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
2 AP	10	329.35304524	76.01784938	24.03895469	206.53272681	437.76851150
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472

Variances	T	DF	Prob>|T|
Unequal	2.1793	27.4	0.0381
Equal	1.8241	28.0	0.0788

For H0: Variances are equal, F' = 3.11 DF = (19,9) Prob>F' = 0.0852

Bartlett's Chi-Square - Test for Homogeneity

TTEST PROCEDURE

Variable: DPM

TREATMEN	N	Mean	Std Dev	Std Error	Minimum	Maximum
AP-50mg/Kg	9	113.08225689	104.55524420	34.85174807	13.96577755	320.96124811
DNCB	20	245.62712343	134.02013272	29.96781271	47.93658782	596.50226472

Variances	T	DF	Prob>|T|
Unequal	-2.8836	19.7	0.0093
Equal	-2.6206	27.0	0.0142

For H0: Variances are equal, F' = 1.64 DF = (19,8) Prob>F' = 0.4806

One-Way ANOVA on EPA / Air Force 90 Day LLNA

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TREATMEN	6	0.02 AP 0.06 AP 0.2 AP 2 AP AP-50mg/Kg DNCB

Number of observations in data set = 70

NOTE: Due to missing values, only 69 observations can be used in this analysis.

One-Way ANOVA on EPA / Air Force 90 Day LLNA

General Linear Models Procedure

Dependent Variable: DPM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	605322.91968788	121064.58393758	8.47	0.0001
Error	63	900229.80612538	14289.36200199		
Corrected Total	68	1505552.72581326			

R-Square	C.V.	Root MSE	DPM Mean
0.402060	40.05818	119.53811945	298.41128715

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREATMEN	5	605322.91968788	121064.58393758	8.47	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREATMEN	5	605322.91968788	121064.58393758	8.47	0.0001

One-Way ANOVA on EPA / Air Force 90 Day LLNA

General Linear Models Procedure

Bartlett's Test for Equality of DPM Variance

Source	DF	Chisq Value	Prob>Chisq
TREATMEN	5	12.0417	0.0342

One-Way ANOVA on EPA / Air Force 90 Day LLNA

General Linear Models Procedure

Dunnett's T tests for variable: DPM

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 63 MSE= 14289.36
Critical Value of Dunnett's T= 2.618

Comparisons significant at the 0.05 level are indicated by '***'.

TREATMEN Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.06 AP	- DNCB	37.31	158.53	279.75	***
0.2 AP	- DNCB	36.18	157.39	278.61	***
0.02 AP	- DNCB	-37.36	83.85	205.07	
2 AP	- DNCB	-37.49	83.73	204.94	
AP-50mg/Kg	- DNCB	-258.17	-132.54	-6.92	***

Non-parametric One-Way ANOVA (Kruskal-Walis) on EPA / Air Force 90 Day LLNA

N P A R 1 W A Y P R O C E D U R E

Wilcoxon Scores (Rank Sums) for Variable DPM
Classified by Variable TREATMEN

TREATMEN	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
0.02 AP	10	400.500000	350.0	58.6651838	40.0500000
0.06 AP	10	519.000000	350.0	58.6651838	51.9000000
0.2 AP	10	466.500000	350.0	58.6651838	46.6500000
2 AP	10	402.000000	350.0	58.6651838	40.2000000
AP-50mg/	9	103.000000	315.0	56.1243481	11.4444444
DNCB	20	524.000000	700.0	75.6079509	26.2000000

Average Scores Were Used for Ties

Kruskal-Wallis Test (Chi-Square Approximation)
CHISQ = 28.029 DF = 5 Prob > CHISQ = 0.0001

Non-parametric One-Way ANOVA (Kruskal-Walis) on EPA / Air Force 90 Day LLNA
Testing the significance of dose in order of
DNCB, and AP: 0.02, 0.06, 0.2, 2 and 50

STATISTICS FOR TABLE OF DOSE BY DPM

Jonckheere-Terpstra Test

Statistic = 979.500 Standardized = 0.101
Prob (Right-sided) = 0.460
Prob (Two-sided) = 0.920

Effective Sample Size = 69
Frequency Missing = 1

Non-parametric One-Way ANOVA (Kruskal-Walis) on EPA / Air Force 90 Day LLNA
Testing the significance of dose in order of
DNCB, and AP: 0.02, 0.06, 0.2, 2 - TAKING OUT 50

STATISTICS FOR TABLE OF DOSE BY DPM

Jonckheere-Terpstra Test

Statistic = 921.500 Standardized = 2.913
Prob (Right-sided) = 0.002
Prob (Two-sided) = 0.004

Sample Size = 60

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM	SE	SI	GROUP_SI	SE_1	DOSE
1	0.02 AP	390.8	490.839	329.4794036	27.37	18.1943793911	12.213115	1.014	0.02
2	0.02 AP	296.8	372.0622		.	13.7915690867	.	.	0.02
3	0.02 AP	284.8	356.8992		.	13.2295081967	.	.	0.02
4	0.02 AP	212.8	265.9212		.	9.85714285714	.	.	0.02
5	0.02 AP	187.8	234.3316		.	8.68618266979	.	.	0.02
6	0.02 AP	149.8	186.3154		.	6.90632318501	.	.	0.02
7	0.02 AP	293.8	368.2714		.	13.6510538642	.	.	0.02
8	0.02 AP	269.8	337.9454		.	12.5269320843	.	.	0.02
9	0.02 AP	304.8	382.1708		.	14.1662763466	.	.	0.02
10	0.02 AP	239.8	300.0379		.	11.1217798595	.	.	0.02
11	0.06 AP	248.8	311.4102	404.1571898	23.64	11.5433255269	14.981265	0.876	0.06
12	0.06 AP	399.8	502.2113		.	18.6159250585	.	.	0.06
13	0.06 AP	247.8	310.1466		.	11.4964871194	.	.	0.06
14	0.06 AP	410.8	516.1107		.	19.131147541	.	.	0.06
15	0.06 AP	299.8	375.8529		.	13.9320843091	.	.	0.06
16	0.06 AP	332.8	417.5512		.	15.4777517564	.	.	0.06
17	0.06 AP	358.8	450.4043		.	16.6955503513	.	.	0.06
18	0.06 AP	352.8	442.8228		.	16.4145199063	.	.	0.06
19	0.06 AP	261.8	327.8367		.	12.1522248244	.	.	0.06
20	0.06 AP	308.8	387.2252		.	14.3536299766	.	.	0.06
21	0.2 AP	582.8	733.4471	403.0199646	57.91	27.18735363	14.93911	2.146	0.20
22	0.2 AP	424.8	533.8009		.	19.7868852459	.	.	0.20
23	0.2 AP	193.8	241.9131		.	8.96721311475	.	.	0.20
24	0.2 AP	304.8	382.1708		.	14.1662763466	.	.	0.20
25	0.2 AP	480.8	604.5615		.	22.4098360656	.	.	0.20
26	0.2 AP	353.8	444.0864		.	16.4613583138	.	.	0.20
27	0.2 AP	313.8	393.5431		.	14.5878220141	.	.	0.20
28	0.2 AP	96.8	119.3455		.	4.42388758782	.	.	0.20
29	0.2 AP	225.8	282.3477		.	10.4660421546	.	.	0.20
30	0.2 AP	235.8	294.9836		.	10.9344262295	.	.	0.20
31	2 AP	209.8	262.1304	329.3530452	24.04	9.71662763466	12.208431	0.891	2.00
32	2 AP	165.8	206.5327		.	7.65573770492	.	.	2.00
33	2 AP	263.8	330.3639		.	12.2459016393	.	.	2.00
34	2 AP	204.8	255.8125		.	9.48243559719	.	.	2.00
35	2 AP	297.8	373.3258		.	13.8384074941	.	.	2.00
36	2 AP	348.8	437.7685		.	16.2271662763	.	.	2.00
37	2 AP	281.8	353.1084		.	13.0889929742	.	.	2.00
38	2 AP	344.8	432.7142		.	16.0398126464	.	.	2.00
39	2 AP	280.8	351.8448		.	13.0421545667	.	.	2.00
40	2 AP	231.8	289.9292		.	10.7470725995	.	.	2.00
41	AP-50mg/Kg	170.8	214.0161	113.0822569	34.85	136.6875	72.223214	.	50.00
42	AP-50mg/Kg	39.8	49.19477		.	31.4196428571	.	.	50.00
43	AP-50mg/Kg	57.8	71.84197		.	45.8839285714	.	.	50.00
44	AP-50mg/Kg	.	.	OMIT 1-4	.	.	.	.	50.00
45	AP-50mg/Kg	154.8	193.8853		.	123.830357143	.	.	50.00
46	AP-50mg/Kg	27.8	34.09663		.	21.7767857143	.	.	50.00
47	AP-50mg/Kg	50.8	63.03473		.	40.2589285714	.	.	50.00
48	AP-50mg/Kg	45.8	56.74383		.	36.2410714286	.	.	50.00

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM	SE	SI	GROUP_SI	SE_1	DOSE
49	AP-50mg/Kg	11.8	13.96578		.	8.91964285714	.	.	50
50	AP-50mg/Kg	255.8	320.9612		.	204.991071429	.	.	50
51	DNCB	262.8	329.1003	234.9633561	28.52	12.1990632319	8.7096019	1.057	0
52	DNCB	138.8	172.416		.	6.39110070258	.	.	0
53	DNCB	108.8	134.5085		.	4.98594847775	.	.	0
54	DNCB	214.8	268.4483		.	9.95081967213	.	.	0
55	DNCB	148.8	185.0518		.	6.85948477752	.	.	0
56	DNCB	323.8	406.1789		.	15.056206089	.	.	0
57	DNCB	153.8	191.3697		.	7.09367681499	.	.	0
58	DNCB	158.8	197.6876		.	7.32786885246	.	.	0
59	DNCB	119.8	148.4079		.	5.50117096019	.	.	0
60	DNCB	252.8	316.4645		.	11.7306791569	.	.	0
61	DNCB	38.8	47.93659	256.2908908	54.34	30.6160714286	163.6875	34.71	0
62	DNCB	132.8	166.2053		.	106.151785714	.	.	0
63	DNCB	174.8	219.0488		.	139.901785714	.	.	0
64	DNCB	62.8	78.13286		.	49.9017857143	.	.	0
65	DNCB	125.8	157.3981		.	100.526785714	.	.	0
66	DNCB	384.8	483.2662		.	308.651785714	.	.	0
67	DNCB	253.8	318.4449		.	203.383928571	.	.	0
68	DNCB	474.8	596.5023		.	380.973214286	.	.	0
69	DNCB	202.8	254.2778		.	162.401785714	.	.	0
70	DNCB	192.8	241.696		.	154.366071429	.	.	0
71	DNCB + CP	73.8	90.28304	213.6087945	37.51	3.34660421546	7.9180328	1.39	1000
72	DNCB + CP	284.8	356.8992		.	13.2295081967	.	.	1000
73	DNCB + CP	79.8	97.86454		.	3.62763466042	.	.	1000
74	DNCB + CP	109.8	135.772		.	5.03278688525	.	.	1000
75	DNCB + CP	138.8	172.416		.	6.39110070258	.	.	1000
76	DNCB + CP	83.8	102.9189		.	3.8149882904	.	.	1000
77	DNCB + CP	214.8	268.4483		.	9.95081967213	.	.	1000
78	DNCB + CP	189.8	236.8587		.	8.77985948478	.	.	1000
79	DNCB + CP	183.8	229.2772		.	8.49882903981	.	.	1000
80	DNCB + CP	354.8	445.35		.	16.5081967213	.	.	1000
81	DNCB + CP	178.8	224.0815	204.2302746	22.22	143.116071429	217.45323	.	1000
82	DNCB + CP	131.8	164.9472		.	105.348214286	.	.	1000
83	DNCB + CP	117.8	147.3327		.	94.0982142857	.	.	1000
84	DNCB + CP	272.8	342.3503		.	218.651785714	.	.	1000
85	DNCB + CP	.	.	OMIT 7-5	.	.	.	.	1000
86	DNCB + CP	86.8	108.3291		.	69.1875	.	.	1000
87	DNCB + CP	182.8	229.1142		.	146.330357143	.	.	1000
88	DNCB + CP	176.8	221.5652		.	141.508928571	.	.	1000
89	DNCB + CP	178.8	224.0815		.	143.116071429	.	.	1000
90	DNCB + CP	140.8	176.2708		.	112.580357143	.	.	1000
91	Vehicle	12.8	13.20445	26.9775082	8.475	.	.	.	-1
92	Vehicle	71.8	87.75588		.	.	.	.	-1
93	Vehicle	24.8	28.36745		.	.	.	.	-1
94	Vehicle	11.8	11.94086		.	.	.	.	-1
95	Vehicle	18.8	20.78595		.	.	.	.	-1
96	Vehicle	9.8	9.413697		.	.	.	.	-1

Print of the dataset

OBS	TREATMEN	CPM	DPM	MEAN_DPM	SE	SI	GROUP_SI	SE_1	DOSE
97	Vehicle	7.8	6.88653		.	.	.	.	-1
98	Vehicle	51.8	62.48421		.	.	.	.	-1
99	Vehicle	10.8	10.67728		.	.	.	.	-1
100	Vehicle	16.8	18.25878		.	.	.	.	-1
101	Vehicle	0	0	1.5657328	1.55	0	1	0.939	-1
102	Vehicle	0	0		.	0	.	.	-1
103	Vehicle	11.8	13.96578		.	8.91964285714	.	.	-1
104	Vehicle	0.8	0.125818		.	0.08035714286	.	.	-1
105	Vehicle	0	0		.	0	.	.	-1
106	Vehicle	0	0	(-0.9 values a	.	0	.	.	-1
107	Vehicle	0	0		.	0	.	.	-1
108	Vehicle	0	0		.	0	.	.	-1
109	Vehicle	0	0		.	0	.	.	-1
110	Vehicle	.	.	OMIT 4-5	.	.	.	.	-1